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THE METABOLISM AND SOME EFFECTS OF p,p' -DDT
IN JAPANESE QUAIL

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

FALL, 1972

UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The metabolism and some effects of *p,p'*-DDT in Japanese quail" submitted by William Allan McBlain in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

Some effects of *p,p'*-DDT on 34 laying Japanese quail (*Coturnix coturnix japonica*) were investigated over a period of 101 days. A fixed dosage of 9 mg/kg (based on bird body weight) of the DDT was administered daily in capsules and both reproductive and metabolic parameters monitored.

This dosage and the resultant body residues of DDT and metabolites, DDD and DDE, had no effect on live body weight, liver and uropygial gland weights, egg production, egg weight or eggshell thickness.

Eggs, livers and fat tissues exhibited a general pattern of DDT and metabolite residue levels as follows: there was an increase of residues early in the study; a maximum residue level was reached midway through the study; and a reduction of residue levels was observed late in the study. The birds seemingly adapted to the pesticide such that DDT and metabolite excretion equalled or surpassed DDT intake, as magnification of residues in tissues was apparently limited. Relatively low levels of DDT, DDD and DDE excreted from 5 birds near the end of the study suggested conversions to undetected metabolites.

Attempts were made to correlate total residue levels of DDT and metabolites in eggs, livers, fat and uropygial glands of 6 birds sacrificed late in the study. Only the correlation of fat levels to egg levels was significant.

ACKNOWLEDGEMENTS

I wish to express my thanks to my supervisors, Dr. V. Lewin and Dr. F. H. Wolfe, for their advice, assistance and encouragement during the study and as well for their critical reviews of the manuscript.

I should further like to thank Dr. W. C. Mackay of my committee for his critical review of the manuscript.

The assistance of Mr. B. C. Switzer with the gas chromatography is gratefully acknowledged.

I wish to acknowledge the financial assistance in the form of National Research Council Grants to Dr. V. Lewin and Dr. F. H. Wolfe, and National Research Council Scholarships (1969, 1971-72) and a National Research Council Bursary (1970) to myself.

I wish also to thank Miss L. Vanderstam for assistance with the original typing of the manuscript.

TABLE OF CONTENTS

ABSTRACT

ACKNOWLEDGEMENTS

LIST OF TABLES

LIST OF FIGURES

	Page
INTRODUCTION	1
MATERIALS AND METHODS	11
Experimental Design	11
Birds	12
Capsules	14
Gas Liquid Chromatography	15
DDT and Metabolite Residues	16
RESULTS	18
Bird Weights	18
Liver and Uropygial Gland Weights	20
Egg Production	21
Egg Weight	21
Eggshell Thickness	23
DDT and Metabolite Residues in Eggs	25
Liver	25
Fat	28
Uropygial Glands and Feces	30
Individual DDT-Treated Bird Parameters	30

	Page
Fate of the DDT	39
Gas Chromatograms	41
Bird Mortality	41
DISCUSSION	45
SUMMARY	50
LITERATURE CITED	52
APPENDIX 1. List of companies supplying chemicals, equipment and materials for this study	57
2. Formula used to calculate tissue residues in parts per million (ppm) or milligrams per kilogram (mg/kg)	58
3. Analysis of quail feed	60
4. The modified cleanup method of Langlois-Stemp-Liska (Bonelli, 1966)	61
5. On-column extraction-cleanup method for animal fat (Cahill <i>et al.</i> , 1970)	62

LIST OF TABLES

Table	Page
1. Mean liver weights of birds expressed in grams and as a percentage of the body weight at sacrifice	20
2. Mean uropygial gland weights of birds expressed in grams and as a percentage of the body weight at sacrifice	20
3. Mean weight of eggs from a random prestudy sample, from selected DDT-treated birds during the study and for a poststudy group of younger birds	23
4. Mean thickness of eggshells from a random prestudy sample, all prestudy experimental birds, selected DDT-treated birds before and during the study and a poststudy group of younger birds	24
5. DDT, DDE and DDD residue levels in livers, fat, uropygial glands and last eggs before sacrifice of DDT-treated birds of cages 37, 40, 41, 43, 44 and 34	38
6. Fate of total ingested <i>p,p'</i> -DDT in 6 DDT-treated birds sacrificed near the end of the study	40
7. DDT intake and excretion for the 24 hr period prior to sacrifice of 6 DDT-treated birds near the end of the study	42

LIST OF FIGURES

Figure	Page
1. Weekly mean bird weights expressed as a percentage of the mean weights at the beginning of the study. The number of birds remaining at each weighing is also included	19
2. Mean number of eggs laid per bird per day by 10-day periods. Numbers of birds remaining at the end of each 10-day interval and standard deviations are also included	22
3. Mean DDT and DDE residues in eggs of DDT-treated birds by 10-day intervals. Standard deviations and sample sizes are included	26
4. Residues of DDT, DDE and DDD in livers of DDT-treated birds sacrificed periodically during the study	27
5. Residues of DDT and DDE in omental fat of selected DDT-treated birds sacrificed during the study	29
6. Parameters of the DDT-treated bird of cage 37. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time	31
7. Parameters of the DDT-treated bird of cage 40. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time	32
8. Parameters of the DDT-treated bird of cage 41. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time	33
9. Parameters of the DDT-treated bird of cage 43. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time	34

Figure	Page
10. Parameters of the DDT-treated bird of cage 44. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time	35
11. Parameters of the DDT-treated bird of cage 34. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time	36
12. Eggshell thickness of the control bird of (a) cage 6 and (b) cage 10 plotted vs time	37
13. Typical gas chromatograms of (a) a 10^{-8} g/ml solution of standards, (b) an egg extract, (c) a liver extract, (d) a fat extract, (e) a uropygial gland extract, and (f) a feces extract	43

INTRODUCTION

Reports in recent years have indicated that DDT and its metabolites are major global chemical pollutants (Edwards, 1970). Though synthesized earlier, DDT received much usage during and following World War II as a cheap, persistent insecticide effective against the insect vectors of typhus and malaria as well as many agricultural pests (Rudd, 1964). When used as an insecticide, DDT is generally a technical grade formulation consisting mostly (about 70%) of *p,p'*-DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) plus *o,p'*-DDT (1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane), and *o,o'*-DDT (1,1,1-trichloro-2,2-bis(*o*-chlorophenyl)ethane) and inert ingredients (Azevedo, 1965; Stecher, 1968). Also present in some preparations are *p,p'*-DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) and *p,p'*-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) (Gill, 1970). DDD and DDE, both common environmental pollutants, are also biological metabolites of DDT (Risebrough *et al.*, 1970; Rudd, 1964). While residues of *p,p'*-DDT are commonly reported in environmental studies *o,p'*-DDT is rarely found (Cooke, 1970; Lamont *et al.*, 1970) possibly due to its conversion to hydroxy, methoxy or other undetected metabolites (Bitman *et al.*, 1971).

As the reviews by O'Brien (1967) and St. Omer (1970) have pointed out much has yet to be learned about the metabolism and mode of action of DDT. Metabolic routes for *p,p'*-DDT vary for different organisms but in birds the main metabolites are DDD and DDE (Menzie, 1969). Of these DDE is usually found far in excess of either DDD or DDT in environmental

avian studies and may result from metabolism of DDT or direct ingestion of DDE (Risebrough *et al.*, 1970). Although other metabolites of DDT exist (Menzie, 1969) they are not usually reported in wildlife studies for several reasons: they are present in very small quantities; they are not present in the tissues generally examined for residues (eggs, muscle, liver, brain or fat); or the analytical techniques generally used do not detect them. The present study is concerned only with *p,p'*-DDT and its major metabolites, DDD and DDE.

The access route of DDT to an animal under natural conditions and in most laboratory studies is the oral route. Upon entering the digestive tract of the animal DDT becomes subject to one or more of the following fates: it may be excreted immediately without alteration; it may be absorbed into the animal tissue without alteration; or it may be metabolized in the gut and then absorbed or excreted. Once absorbed the DDT may be transported, stored, metabolized and/or excreted. Blood is the prime vehicle of transport in the vertebrate body and as Apple *et al.* (1970) have reported appropriate blood analysis gives a good index of recent exposure to DDT and its metabolites. Due to its relatively high solubility in lipids and low solubility in aqueous solutions, DDT probably moves in the blood complexed with lipids or lipoproteins (Ecobichon and Saschenbrecker, 1968). Furthermore, when detected in tissues such as muscle, brain or eggs DDT is also associated with the portions of these tissues having a high lipid content. Thus adipose tissue in general constitutes the main storage site for the hydrophobic DDT.

Metabolism of DDT within the vertebrate body occurs mainly in

the liver as a result of the induction of liver microsomal enzymes (Street, 1969). It would seem that the hepatic enzymes induced are probably polyfunctional in that they may be induced by and act on other chemicals or drugs (Alary *et al.*, 1971). It is this effect on other chemicals which has led to the theory that DDT may cause excessive metabolism of important steroids by inducing these hepatic microsomal enzymes. Some metabolism may also take place in the gut before absorption and metabolites may circulate in the enterohepatic pathway (Roan *et al.*, 1971).

Excretion of DDT and metabolites probably occurs mainly via the kidney (Tocci *et al.*, 1969) and gut (either fecal or biliary routes), although in birds the uropygial gland (especially in birds of aquatic or marine habitats where it is most developed) and eggs may represent alternate excretory routes.

Given a steady dietary intake of DDT, it has been suggested that an animal will exhibit a plateau level of DDT and metabolites in its tissues (Dustman and Stickel, 1969). A plateau of residues results in a tissue when DDT intake is equal to or in equilibrium with metabolism and/or excretion of DDT from the tissue. The relatively constant level of DDT and DDE found in humans since the early 1950's is evidence for such a phenomenon (Davies *et al.*, 1969; Durham, 1969). A study of DDE in rat adipose tissue (Durham, 1969) and of DDT and DDE in hen eggs (Cummings *et al.*, 1965) has given more credence to this idea. It has further been postulated that if a plateau level of DDT residues is reached in the body and the appropriate equilibria set up with respect to absorption, transport, metabolism and excretion, various body

tissues may have residue levels which can be correlated. Thus it has been assumed that the residues in a bird's eggs may be an indicator of residues in other tissues of the female (Cade *et al.*, 1971).

DDE in birds and their eggs has been implicated as a cause of shell thinning and subsequent reproductive failure in certain birds of prey such as the peregrine falcon (*Falco peregrinus*) (Ratcliffe, 1967; Hickey, 1969), the osprey (*Pandion haliaetus*) (Ames, 1966; Hickey and Anderson, 1968) and the bald eagle (*Haliaeetus leucocephalus*) (Krantz *et al.*, 1970). Birds such as the brown pelican (*Pelecanus occidentalis*) and herring gull (*Larus argentatus*) associated with marine or aquatic habitats have also had instances of low reproductive success which have been correlated with high DDE residues in their eggs (Risebrough *et al.*, 1970; Hickey and Anderson, 1968). Thus the persistent pesticide DDT (and/or its metabolite DDE) is thought to be exerting sublethal effects on these vertebrate populations.

The mode of action of DDT or its metabolites to bring about these reported effects is at best uncertain. Several hypotheses exist as reviewed by Peakall (1970) and Risebrough (1970) which might be divided into two categories, enzymatic and hormonal effects.

Enzymatic effects include possible effects of DDT (or DDE) on ATPase enzymes in the intestinal or shell gland membranes. These enzymes are essential for the active transport of calcium from the gut to the body and also from the body (shell gland) to the eggshell. There may also be a direct inhibition of carbonic anhydrase, the enzyme necessary for calcium release from medullary bone and for calcium release in the shell gland (Taylor, 1970) although recent studies by

Poeker *et al.* (1971) indicate that this inhibition does not occur.

Hormonal effects include the induction of hepatic microsomal enzymes which could metabolize steroids such as estrogens and testosterone and delay oviposition, alter calcium metabolism and eggshell formation or alter breeding behavior. For Japanese quail (*Coturnix coturnix*), however, hepatic microsomal epoxidase activity was depressed rather than increased after 23 weeks of exposure to 100 ppm *p,p'*-DDT in the feed (Gillett and Arscott, 1969). The liver microsomes may affect vitamin D₃, a steroid thought necessary for the synthesis of a calcium-binding protein and necessary for the active transport of calcium. Nowicki *et al.* (1971) support the concept that DDT affects the biological functions of vitamin D₃ but not its metabolism to active forms. Mimmick-
ing of estrogen or thyroxine by DDT or its metabolites could alter breeding behavior or cause hyperthyroidism respectively. DDT may also affect the thyroid directly (causing hyperthyroidism at low levels by inducing thyroxine production and hypothyroidism at high levels probably as a function of competition with thyroxine for a binding site on blood protein during transport) to bring about changes in several physiological parameters (Jefferies *et al.*, 1971).

Presence of DDE and a low reproductive success in wild avian populations are not always correlative. Switzer *et al.* (1971) showed that DDE levels in eggs of common terns (*Sterna hirundo*) did not correlate with eggshell thickness and that the low reproductive success observed was due to other environmental factors. It is possible that other investigators may not have isolated the real cause(s) of low reproductive successes they observed.

Simple laboratory feeding studies designed to investigate the

relationship between dietary DDT (or metabolites) and reproductive parameters in birds (shell thinning, fertility, hatchability, etc.) have generally failed to either produce the degree of effects found in wildlife studies or elucidate the mechanisms involved in producing the effects. Porter and Wiemeyer (1969) conducted feeding experiments using captive raptors (the most commonly affected group of birds), American sparrow hawks (*Falco sparverius*), but used a mixture of DDT and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene) to achieve shell thinning. The contribution to the thinning by DDT is unknown since dieldrin alone can thin eggshells (Lockie *et al.*, 1969).

Thin eggshells and a lowered reproductive success in terms of young survival to 14 days were later produced in a feeding study employing mallard ducks (*Anas platyrhynchos*). In this case the effects were induced by 10 ppm or 40 ppm DDE in the feed (Heath *et al.*, 1969). More recently Davison and Sell (1972) have criticized this and several similar investigations on the basis of statistical treatment of the data thus making the conclusions somewhat questionable. Work with Bengalese finches (*Lonchura striata*) has revealed that *p,p'*-DDT can cause a delay in ovulation as well as either hypo- or hyperthyroidism. Both of these factors could bring about the effects on reproductive success seen in some wild populations (Jefferies, 1967; Jefferies, 1969; Jefferies *et al.*, 1971).

However, most DDT feeding studies have been carried out using galliform birds since they are relatively cheap and easy to maintain and adapt well to artificial cage and feed conditions. Azevedo (1965) determined that 500 ppm of technical grade DDT in the feed of pheasants

(*Phasianus colchicus*) did not reduce egg production, fertility or hatchability but did cause higher chick mortality up to 46 days of age. Dietary calcium and eggshell thickness were not mentioned.

Because of their widespread use as laboratory animals, another galliform bird, Japanese quail, has been used for a large number of laboratory feeding experiments concerned with the effects of DDT on avian reproduction. Cross *et al.* (1962) reported that technical grade DDT in the feed of Japanese quail at concentrations up to 300 ppm had no effect on egg production, hatchability or egg weight. These results were supported by Ernst (1966) who found that 100, 300 or 500 ppm of technical grade DDT in the feed of this species did not affect egg production, fertility or hatchability but that chick mortality to 13 days after hatching was increased. This latter observation was similar to the reduced survival of chicks to day 7 found by Jones and Summers (1968) who fed 200 ppm of technical grade DDT to quail.

Pure *p,p'*-DDT fed to Japanese quail at 100 or 200 ppm in the feed (with an adequate calcium supply above the 2.5 percent required (Nelson *et al.*, 1964)) caused no changes in mortality of parents, egg production, fertility or hatchability but 400 ppm affected all of these parameters (Smith *et al.*, 1969). Thinned eggshells with a reduced calcium content were produced by Japanese quail fed 100 ppm of either *p,p'*-DDT or *o,p'*-DDT in the feed (Bitman *et al.*, 1969), but all birds in these experiments had an inadequate (0.56 percent) calcium supply in the diet. Bitman *et al.* (1970) proposed an inhibition of shell gland carbonic anhydrase as a possible mechanism for the shell-thinning they reported. Unfortunately, since that time Pocker *et al.* (1971) have shown that the

procedure used to measure the carbonic anhydrase activity may be invalid. It is of interest to the present study that for Japanese quail, breeding birds, heavy birds and females are most resistant to the toxic effects of p,p' -DDT (Gish and Chura, 1970).

It is obvious from the above review that the effects of DDT on avian reproduction as revealed by laboratory studies have not been fully clarified. Furthermore, these studies and metabolic studies (Abou-Donia and Menzel, 1968; Ecobichon and Saschenbrecker, 1968; Bailey *et al.*, 1969) have done little to reveal the fate of DDT in birds which are reproductively active. It was the aim of the present study to determine the effects of DDT on laying Japanese quail with reference to both reproduction and insecticide metabolism.

Studies using technical grade DDT (which may vary in composition) do not indicate which of p,p' -DDT, o,p' -DDT or some other similar constituent or metabolite may be active in bringing about the reported effects. Of the original technical grade DDT, p,p' -DDT and its metabolites, DDE and DDD, are the only compounds which are thought to have environmental significance. Therefore, investigations using pure p,p' -DDT (the most abundant constituent of commercial DDT preparations) or DDE (the common avian DDT metabolite) would seem to have more relevance to the problem of ascertaining the precise effects of these pollutants on avian systems.

Smith *et al.* (1969) while reporting the effects of pure p,p' -DDT on several reproductive parameters of Japanese quail did not give information about eggshell thinning, a phenomenon central to similar environmental studies. And although the study of Bitman *et al.* (1969)

reported eggshell thinning induced by both *o,p'*-DDT and *p,p'*-DDT the results were questionable due to the calcium-deficient diet given to the birds. In neither of the studies was the exact dose of chemical ingested by the birds or its metabolic fate known.

Since the present study was undertaken several reports have been published which relate to the findings presented here. Continuing their work with captive American kestrels (sparrow hawks), Wiemeyer and Porter (1970) induced 10 percent eggshell thinning by feeding 2.8 ppm (wet weight) *p,p'*-DDE in ground meat. Captive black ducks (*Anas rubripes*) fed 10 ppm and 30 ppm *p,p'*-DDE laid eggs with shells thinned by 18 percent and 24 percent at the equator respectively. Duckling survival to 21 days after hatch was also significantly reduced (Longcore *et al.*, 1971). Tucker and Haegele (1970) induced no significant shell thinning in mallard ducks as the result of 10 ppm or 30 ppm of technical grade DDT added to the feed. They did induce 25 percent shell thinning, however, by administering 1000 mg/kg technical grade DDT in a gelatin capsule and suggested that such treatment may parallel environmental stress situations where massive doses of DDT may be released from storage sites.

In contrast to their earlier study Cecil *et al.* (1971) reported that 100 ppm of either *p,p'*-DDT or *p,p'*-DDE in the diet of Japanese quail did not cause shell thinning. There was also no change in egg production or egg weight but a delay in the onset of laying was noted. Similarly, Chang (1970) found that *p,p'*-DDT (100 ppm) and *p,p'*-DDE (200 ppm) in the feed of Japanese quail did not affect bird growth or feed consumption and as well had no effect on egg weight but decreased

eggshell thickness by 5 percent. Nowicki *et al.* (1972) reported that 100 ppm of technical grade DDT in the feed of Japanese quail had no effect on eggshell calcium content, eggshell thickness or eggshell structure.

Wright *et al.* (1972) administered 10 mg/kg *p,p'*-DDT to chickens (*Callus domesticus*) in gelatin capsules. Highest body residues (*p,p'*-DDT, *o,p'*-DDT, DDE, *p,p'*-DDD) occurred in the fat and uropygial gland. Egg yolk residues of *p,p'*-DDT reached a maximum at about 60 days but dropped somewhat at 90 days, whereas DDE residues in the egg yolks were still increasing at 90 days. Davison and Sell (1972) also using chickens reported that 100 or 200 ppm of *p,p'*-DDT in the feed had no effect on egg production, egg weight, dry shell weight, shell thickness or shell calcium.

Pure *p,p'*-DDT was employed in the study reported here and administered to the birds as a known fixed daily dose. The reproductive parameters of egg production, egg weight and eggshell thickness were monitored under the condition of a slight dietary calcium stress. Furthermore, the metabolic fate of the DDT was determined in part by qualitative and quantitative analyses of selected tissues. This combination of reproductive and metabolic investigations allowed a re-evaluation of the previous literature regarding the effects of DDT on Japanese quail.

MATERIALS AND METHODS

Experimental Design

A known daily dose of 9 ppm (based on bird body weight at the beginning of the study) of pure *p,p'*-DDT was administered to 34 individually housed Japanese quail (described below). Given a daily food consumption of 15-19 g per day for a 150 g bird (Cross *et al.*, 1962), the fixed 9 ppm body weight dosage of *p,p'*-DDT compared roughly to that of a diet contaminated with 100 ppm as used by Smith *et al.* (1969) and Bitman *et al.* (1969). Ten birds receiving no DDT served as controls.

Data concerning the reproductive parameters of egg production, egg weight and eggshell thickness were collected to determine if the dosage of DDT and resultant body residues could induce such phenomena as have been reported in wildlife studies.

Both DDT-treated and control birds were sacrificed at intervals throughout the study and the metabolic fate of the *p,p'*-DDT was in part determined by the following investigations: DDT and metabolite residue levels were monitored over 101 days in eggs, livers and fat tissues to test for a plateau level of residues in these tissues and to look for possible correlations between residue levels present; residues in feces and uropygial glands were checked qualitatively to see which chemicals were excreted and if the oily excretory uropygial gland concentrated the lipid-soluble DDT and metabolites; relative amounts of DDT, DDE and DDD determined for the eggs, livers and fat tissues were compared to give some idea of relative conversion rates from the parent *p,p'*-DDT;

monitoring of tissue residues for individual birds also gave an indication of the magnitude of individual variations in metabolism to be expected.

Birds

Forty-four Japanese quail (*Coturnix coturnix japonica*) of about 27 weeks of age were selected from BAS (Bioscience Animal Services)¹ cages where they had been held in groups of 10 females per cage. In all cases heavier birds tended to be selected. All experimental birds were weighed, marked with numbered plastic leg bands and placed in 11 x 10 x 5 inch wire mesh individual cages and given water, grit and feed *ad libitum*. The grit was Canadian Granite Grit No. 1 supplied by Valley Granite Products, and the feed was Turkey Starter mixed by Federated Co-operatives Limited (see Appendix 3) to which had been added 0.48% calcium (Ca) by weight in the form of powdered CaCO_3 to bring the total percent by weight of Ca up to 1.78%. The birds' previous calcium source had been the feed (1.3% by weight) and oyster shell fed *ad libitum*. Gas chromatographic analysis revealed that the feed was contaminated with 0.06 ppm DDE, 0.10 ppm DDD and 0.32 ppm *p,p'*-DDT.

The light regime during the study was 14 hours of light and 10 hours of dark and the temperature was maintained at 73.5-75° F with one exception. Egg production was monitored from the time the birds were put in individual cages to the termination of the experiment. The study was initiated 11 days after the initial selection of the birds and during this time 2 birds laying soft-shelled eggs and 1 laying bloody eggs were

¹See Appendix 1 for a list of complete names and addresses of companies supplying chemicals, equipment and materials for this study.

replaced. Birds were reweighed at the beginning of the study and once a week until their sacrifice.

DDT-treated birds and controls were dosed at the same time each morning. For treatment each bird was held in a dark sock open at either end and a capsule (described below) was wetted in water and placed in the pharynx using forceps. In no case was a capsule rejected during or after treatment and in no case were capsules or capsule remnants found in feces.

Sacrifices (by cervical dislocation) among control birds occurred every 6 days until day 30 and every 12 days thereafter. Similarly, DDT-treated birds were sacrificed every 2 days until day 29 and every 4 days following. The liver, uropygial gland and a sample of visceral fat (omental fat pad) were weighed to the nearest 0.001 g while fresh, and with the previous days' feces were saved frozen in cork-stoppered 6 dram glass vials which had previously been rinsed with 100% ethanol. The vials were determined by gas chromatographic analysis to be free of interfering impurities. All eggs laid during the study were also stored frozen at -15°C . Egg weight and shell thickness data were also gathered. Shell thickness was determined to the nearest 0.001 mm using a dial micrometer described by Lewin (1970). Both ends of the egg and 2 locations on the equator were measured after removal of the 2 shell membranes and a mean thickness value calculated from these measurements. Membranes were peeled from appropriate shell fragments following a brief boiling in tap water and these fragments were dried before measurement.

On day 24 the birds were moved to another room with the same light regime and initially the same temperature as previously described.

On day 26 the temperature dropped to 67° F and was not returned to 73.5° F until day 30 but remained at 73.5° F until day 44 when it dropped to 71° F. The temperature remained low, 71-72° F, until the birds were returned to their original room on day 51. This temperature change combined with the unfamiliar surroundings seemed to stress the birds as witnessed by changes in several parameters (see Results section).

Capsules

99+% pure *p,p'*-DDT (Aldrich Chemical Co.) was prepared for administration to the birds in size 5 gelatin capsules (Eli Lilly and Company). The capsules were prepared by injecting 0.08, 0.09, 0.10 or 0.11 ml of a 15 mg/ml solution of *p,p'*-DDT in acetone (Mallinckrodt Chemical Works Ltd.) into half capsules held in plastic holding blocks in a fume hood. An automatic pipetter (Manostat Corporation) was used for the injections. These capsules contained an amount of DDT which, relative to bird body weight at the beginning of the study, gave a treatment of 9 mg/kg (ppm).

<u>Bird weight (g)</u>	<u>Volume of DDT stock solution (15 mg/ml) in capsule (ml)</u>
112.5-127.0	0.08
127.5-142.0	0.09
142.5-157.0	0.10
157.5-172.0	0.11

Gas chromatographic analyses of 3 samples of prepared capsules without feed revealed that there had been about a 10% loss of DDT from that originally calculated probably due to inert ingredients in the *p,p'*-DDT and a small amount of coevaporation. The loss was accounted

for and the resulting fixed treatment of DDT was about 9 mg/kg. Following evaporation of the acetone, feed (described above) was placed in the capsule half and the capsule reassembled. Capsules were stored in glass vials (described above) at -15° C. Control capsules were prepared by evaporating 0.10 ml acetone from half capsules, adding feed and capping. Gas chromatographic analysis revealed no impurities in the acetone which might affect the birds.

Gas Liquid Chromatography

All residue identification and quantitation analyses for this study were carried out using a Varian Aerograph 600-D gas liquid chromatograph equipped with a 250 microcurie tritium source electron capture detector. A 5-foot glass column packed with a 1:1 mixture of 10% DC-200 and 15% QF-1 on 80/90 mesh Anakrom ABS was used for separation of the compounds and peak heights recorded on a Varian Aerograph Model 20 strip chart recorder were used for quantitations. The carrier gas was purified nitrogen (L grade--Canadian Liquid Air Limited) at a flow rate of 70-85 ml/min. Other operating conditions were as follows: the oven temperature was $175-185^{\circ}$ C; the injector temperature was about 200° C (this model of chromatograph does not monitor the injector temperature); attenuation 4 was most commonly used but 8 and 16 were also employed.

Injections made using a Hamilton 10 μ l (701-N) syringe were always in the range of 2-8 μ l. The detector foil was cleaned either in an ultrasonic cleaner using an alcoholic KOH solution or with Q-tips (Chesebrough-Ponds Ltd.) using a chlorinated cyanuric acid solution (Super Dutch Cleanser--Purex Corporation Ltd.). In both cases the foil was then rinsed in purified methanol (Fisher Scientific Co.). Teflon-

faced injector septa (Varian Aerograph) were used and found to withstand up to about 50 injections.

Standards used were either a 10^{-8} g/ml solution of 9 insecticides or a 10^{-7} or 10^{-8} standard of DDE, DDD and *p,p'*-DDT prepared from Polyscience Corporation quantitation grade standards supplied by Chromatographic Specialties Ltd. Quantitation was done by comparison of unknown peak heights from chromatograms to standard peak heights using the formula given in Appendix 2. An injection of the standard solution was made each time an unknown was quantitated, but if more than 1 unknown was to be injected serially the ratio of unknown injections to standard injections was as high as 4:1.

DDT and Metabolite Residues

DDT and its metabolite residues were extracted from 1 feed sample, 117 eggs, 44 livers, 18 fat samples, 6 uropygial glands and 6 feces samples. A modification of the Langlois-Stemp-Liska technique (Bonelli, 1966) (see Appendix 4) was used for cleanup of the feed, eggs, liver, uropygial glands and feces and quantitation was carried out using the gas liquid chromatograph. About 0.5 g of feed, 5.0 g of egg yolk plus white (blended in an Ivan Sorval omnimixer), whole livers, whole uropygial glands and 0.5 g of dried feces were utilized for residue extractions.

For eggs this cleanup method gave recoveries of 91.1% DDE, 90.7% DDD and 98.5% *p,p'*-DDT. For livers the recoveries were 95.3% DDE, 96.8% DDD and 100.0% *p,p'*-DDT, and for uropygial glands 100.4% DDE, 108.8% DDD and 118.7% *p,p'*-DDT.

Fat samples were cleaned up by the on-column method of Cahill *et al.* (1970) (see Appendix 5) but recovery values were not determined due to problems in spiking fat samples accurately. For this method the Florisil was prepared as described in Appendix 4 and fat samples of 0.174-0.218 g were kept frozen between weighing and cleanup by the use of dry ice.

The columns used for all extractions were pyrex tubes (24 mm o.d. x 600 mm) fitted with coarse fritted glass sieves, teflon stopcocks and removable 1-l reservoirs. Eluates were collected in 1-l round-bottom flasks. All glassware used in the extraction procedures was rinsed with hexane between sample runs. Routine blank extractions verified cleanliness of the glassware and purity of the chemicals.

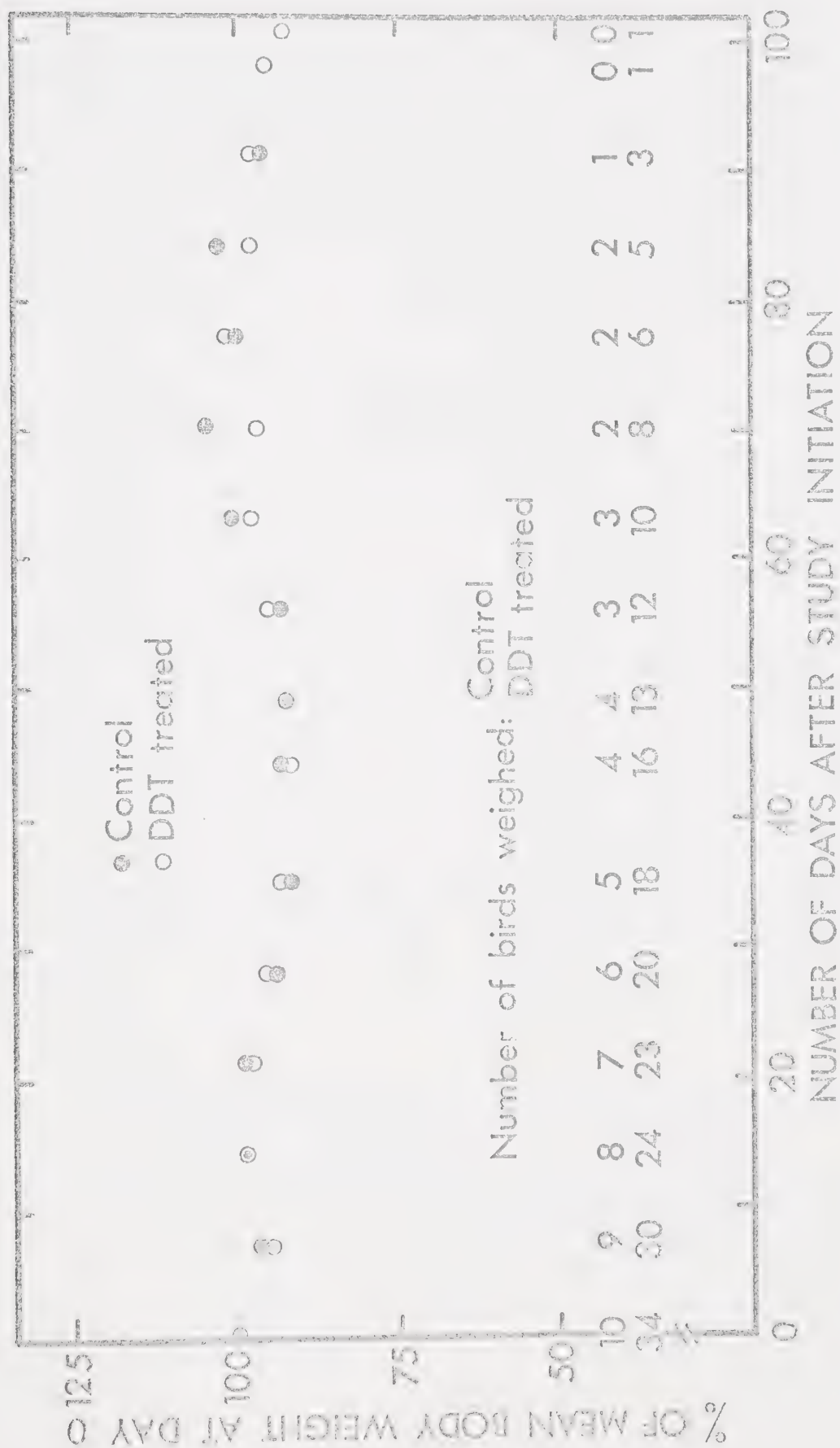
RESULTS

Bird Weights

As can be seen from Figure 1 the mean weekly weights of control birds and DDT-treated birds when expressed as a percentage of the mean weight at day 0 did not differ significantly (Student-t test, $P < .05$). That is, DDT did not affect bird body weights. For the DDT-treated group, however, the mean body weights were decreased significantly (Student-t test, $P < .01$) by day 7 probably as a result of handling the birds for capsule administration. By day 14 the weights did not differ significantly from the day 0 weights (Student-t test, $P < .05$) but by day 28 another significant drop (Student-t test, $P < .05$) had occurred probably as a result of the temperature and room change of day 24 to day 51 mentioned earlier. Control bird body weights showed the same pattern as those of the DDT-treated group but the smaller sample size prevented their changes from being significant (Student-t test, $P < .05$).

As will be seen in Figure 2 egg production at this time was lowered significantly as well and gross examination of the ovaries of birds sacrificed during this period revealed an absence of well-developed preovulatory follicles in 6 of 11 birds. Thus the cessation of laying by many birds at this time could alone account for changes seen in mean body weights. This supports the relationship between body weight and follicular development found by Lewin (1963) for California quail (*Lophortyx californicus*).

Figure 1. Weekly mean bird weights expressed as a percentage of the mean weights at the beginning of the study. The number of birds remaining at each weighing is also included.



Liver and Uropygial Gland Weights

The mean liver weights of birds expressed in grams and as a percentage of body weight at sacrifice are presented in Table 1 and did not differ significantly (Student-t test, $P < .05$) between control and DDT-treated birds.

Table 1. Mean liver weights of birds expressed in grams and as a percentage of the body weight at sacrifice

Group	No. of birds	Mean liver weights	
		g $\pm$ S.D.	% body wt. $\pm$ S.D.
Controls	10	5.80 $\pm$ 1.90	3.76 $\pm$ 1.08
DDT-treated	33	5.22 $\pm$ 1.29	3.60 $\pm$ 0.71

Similarly, the mean uropygial gland weights in Table 2 did not differ significantly (Student-t test, $P < .05$) between control and DDT-treated birds.

Table 2. Mean uropygial gland weights of birds expressed in grams and as a percentage of the body weight at sacrifice

Group	No. of birds	Mean uropygial gland weights	
		g $\pm$ S.D.	% body wt. $\pm$ S.D.
Controls	10	0.229 $\pm$ 0.035	0.15 $\pm$ 0.02
DDT-treated	33	0.221 $\pm$ 0.047	0.15 $\pm$ 0.03

Furthermore there were no changes in liver or uropygial gland weights which could be related to day of sacrifice during the study.

Egg Production

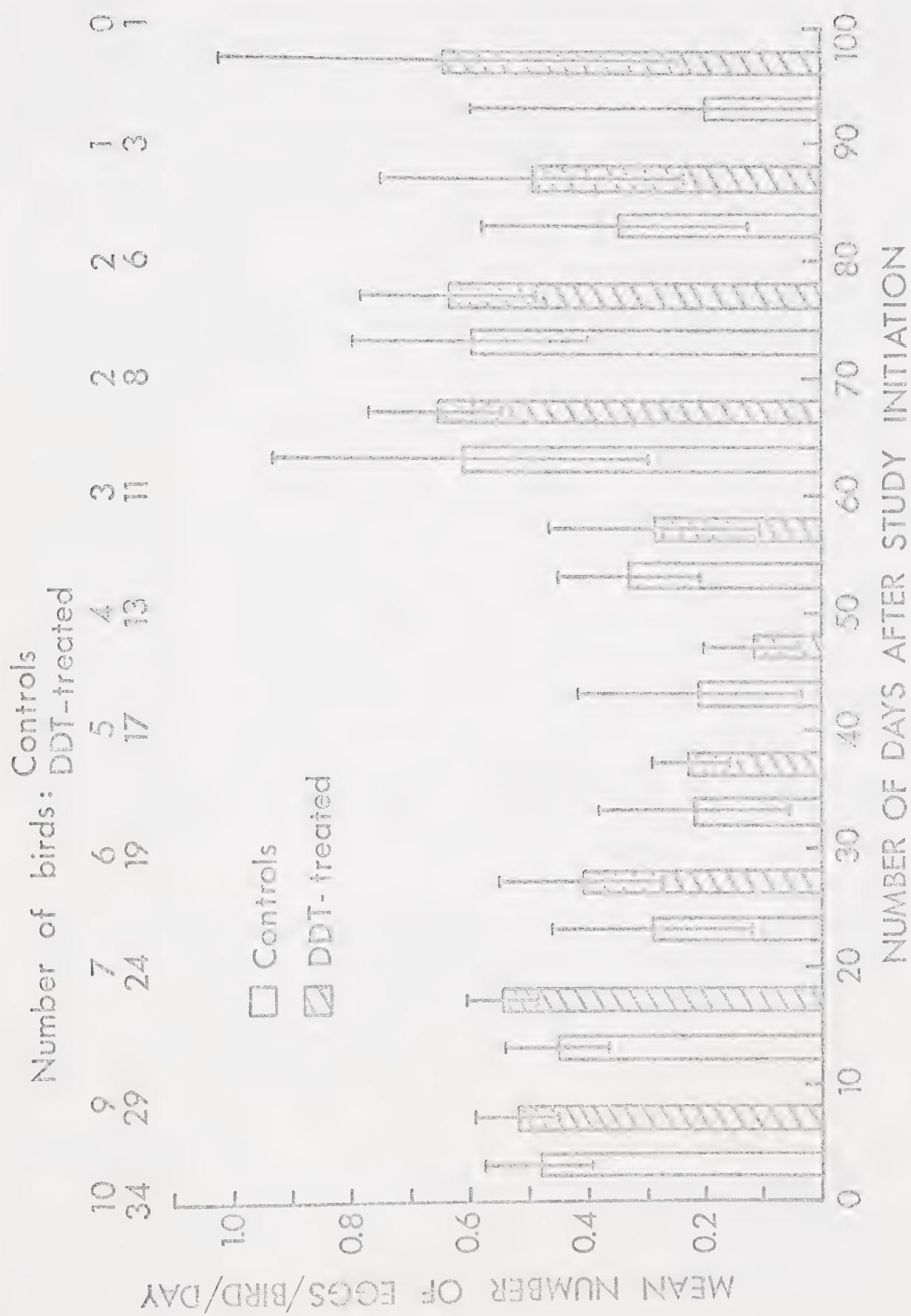
In Figure 2 the mean number of eggs laid per bird per day is presented by 10-day intervals. The 10-day means for control and DDT-treated birds differ significantly (Student-t test, $P < .05$) only for the day 10 to day 20 and for the day 90 to day 100 periods. In both cases the DDT-treated birds laid more eggs but for the 90 to 100 day period the number of birds left is extremely low.

As indicated by the means and standard deviations the initial production of eggs to day 20 was relatively stable for both groups. The effect of the temperature and room change of days 24 to 51 then became obvious as egg production dropped. A recovery period occurred after day 51 and a return to the original egg production of days 0 to 20 was indicated although the low number of birds remaining late in the study added bias to these data. The mean number of eggs laid per bird per day during the entire study did not differ significantly (Student-t test, $P < .05$) between control (0.46 eggs/bird/day) and DDT-treated groups (0.46 eggs/bird/day).

Egg Weight

This study was not ideally suited for the monitoring of egg parameters near its termination due to the low number of control and test birds used and the fact that birds were sacrificed at intervals. Hence for egg weights the mean weight from 8 DDT-treated birds laying from days 69 to 80 was compared to the mean weight of eggs laid by these

Figure 2. Mean number of eggs laid per bird per day by 10-day periods. Numbers of birds remaining at the end of each 10-day interval and standard deviations are also included.



same birds from days 0 to 10. Further comparisons were made to the mean weight of eggs taken at random from the holding cages prior to the study and to the mean egg weight of a group of poststudy 13-week-old birds.

These results are presented in Table 3.

Table 3. Mean weight of eggs from a random prestudy sample, from selected DDT-treated birds during the study and for a poststudy group of younger birds

Group	Period	No. of eggs	Mean egg weight g $\pm$ S.D.
Random	Prestudy	112	8.65 $\pm$ 1.09
DDT-treated	Days 0 to 10	12	8.58 $\pm$ 0.98
	Days 69 to 80	17	8.96 $\pm$ 0.94
Random (13 wk old birds)	Poststudy	56	9.24 $\pm$ 1.06*

*Significantly greater than the first mean presented (Student-t test, $P < .01$).

Only the mean egg weight of the poststudy group of younger birds differed significantly (Student-t test, $P < .01$) from the mean egg weight of the prestudy group. DDT did not alter egg weight in these birds but the younger birds laid heavier eggs.

Eggshell Thickness

For the reasons mentioned above the mean eggshell thickness of eggs from 8 DDT-treated birds laying from days 69 to 80 was compared to the mean eggshell thickness of eggs laid by these same birds between

days -3 and -1. Further comparisons were made to the mean eggshell thickness of eggs from birds of the holding cages prior to the study, birds of all study cages prior to the study, and poststudy 13-week-old birds. These results appear in Table 4.

Table 4. Mean thickness of eggshells from a random prestudy sample, all prestudy experimental birds, selected DDT-treated birds before and during the study and a poststudy group of younger birds

Group	Period	No. of eggs	Mean shell thickness mm $\pm$ S.D.
Random	Prestudy	69	0.157 $\pm$ 0.016
All birds	Days -2 to -1	55	0.167 $\pm$ 0.013*
DDT-treated	Days -3 to -1	10	0.165 $\pm$ 0.017
	Days 69 to 80	16	0.177 $\pm$ 0.010*
Random (13 wk old birds)	Poststudy	56	0.169 $\pm$ 0.012*

*Significantly greater than the prestudy mean (Student-t test, $P < .01$).

The prestudy mean for eggshell thickness is significantly less (Student-t test, $P < .01$) than that of all other groups except the mean of days -3 to -1 for cages 34, 37 and 39-44. This may have been due to the different calcium source for the prestudy group. No other significant differences exist between the means presented. Therefore, DDT did not cause eggshell thinning in these birds. Further evidence for this will be seen when individual bird parameters are discussed (Figures 6 to 11).

DDT and Metabolite Residues in Eggs

The mean residue levels of DDE and *p,p'*-DDT in eggs of DDT-treated birds are presented by 10-day intervals in Figure 3. DDD, although present in eggs, was found to be less than 1 ppm and was not quantitated due to interference with the far more concentrated DDT as the 2 compounds eluted from the gas chromatograph. Both DDE and DDT increased in the eggs until the day 30 to day 40 interval and then remained relatively uniform. Between the day 60 to day 70 interval and the day 80 to day 90 interval a significant (Student-t test, $P < .01$) drop in mean levels of both chemicals was noted indicating that a maximum mean level had been reached. Throughout the study there was a general increase in the ratio of DDE to DDT present in the eggs. A more detailed presentation of residue levels in individual eggs will be seen when individual bird parameters are discussed (Figures 6 to 11). Eggs laid by control birds during the course of the study exhibited a low mean of 0.58 ppm of total residues with a standard deviation of 0.95 ppm for 12 eggs. A typical gas chromatogram for egg residues appears in Figure 13.

DDT and Metabolite Residues in Livers

Figure 4 displays the residue levels of *p,p'*-DDT, DDE and DDD in livers of birds sacrificed at intervals during the study. Due to the fact that only 1 bird was sacrificed at a given time much individual variation probably accounts for the irregular pattern of residue levels seen in this tissue. There was an increase in residues of all three chemicals to day 11 but after this time the pattern became less clear. In general, however, it can be seen that the levels of DDT and its metabolites did not rise continually throughout the study and were data

Figure 3. Mean DDT and DDE residues in eggs of DDT-treated birds by 10-day intervals. Standard deviations and sample sizes are included.

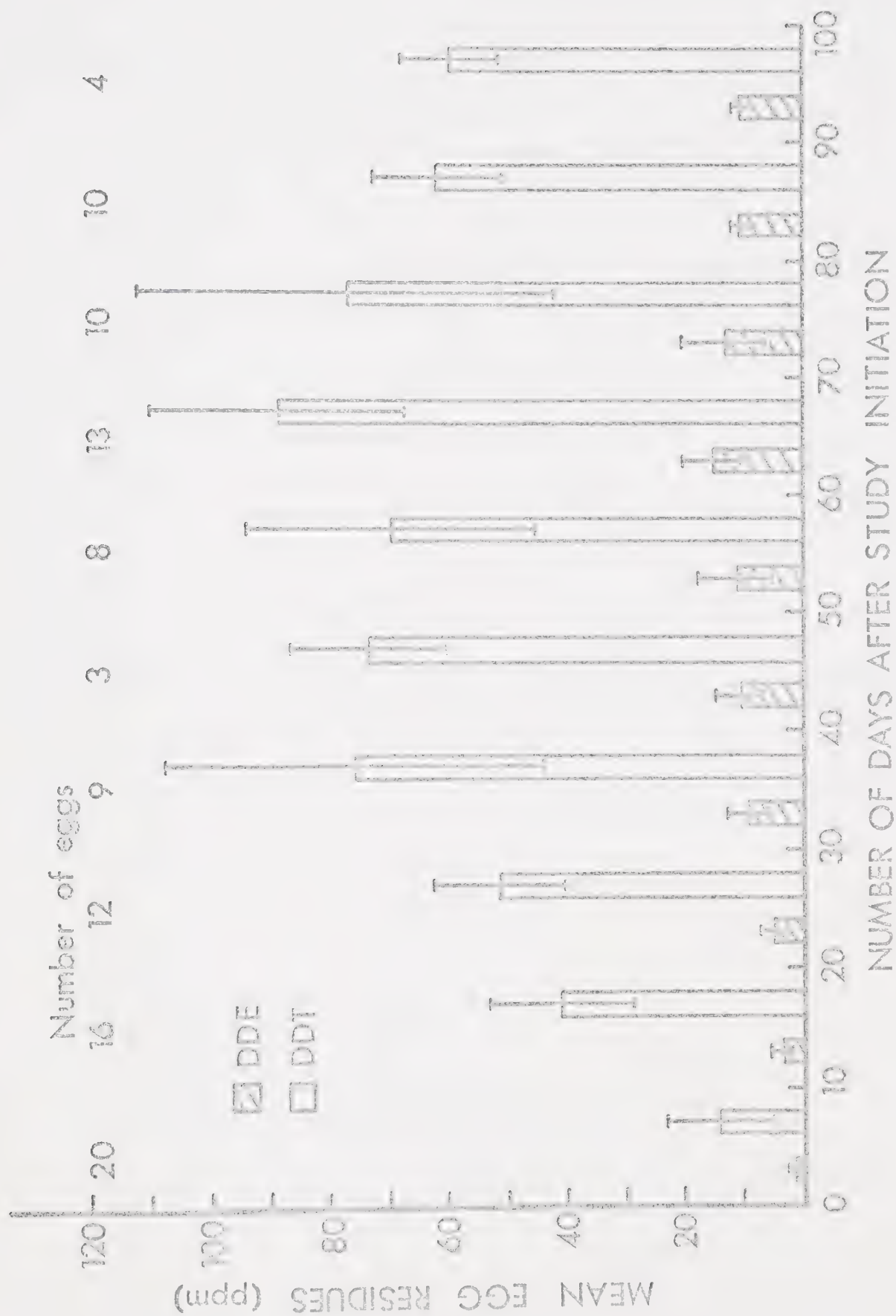
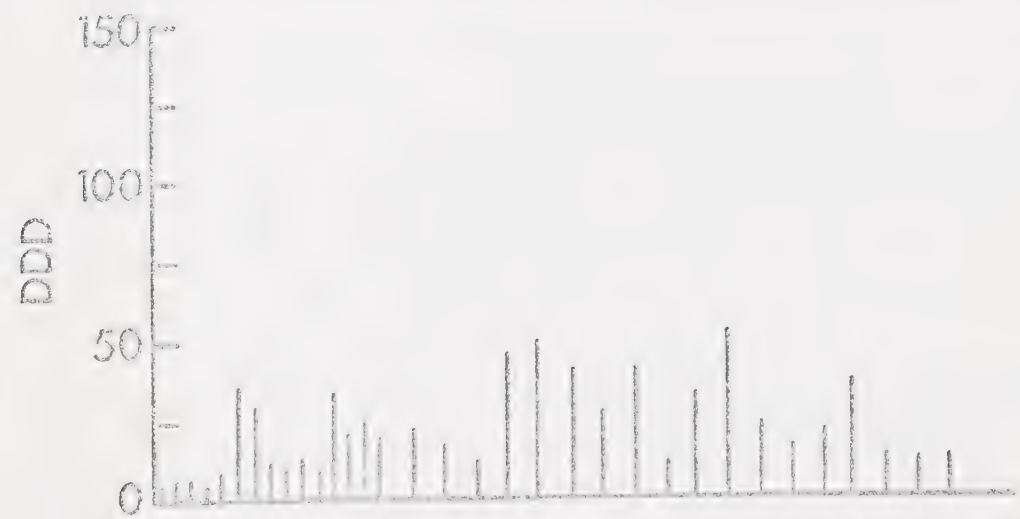
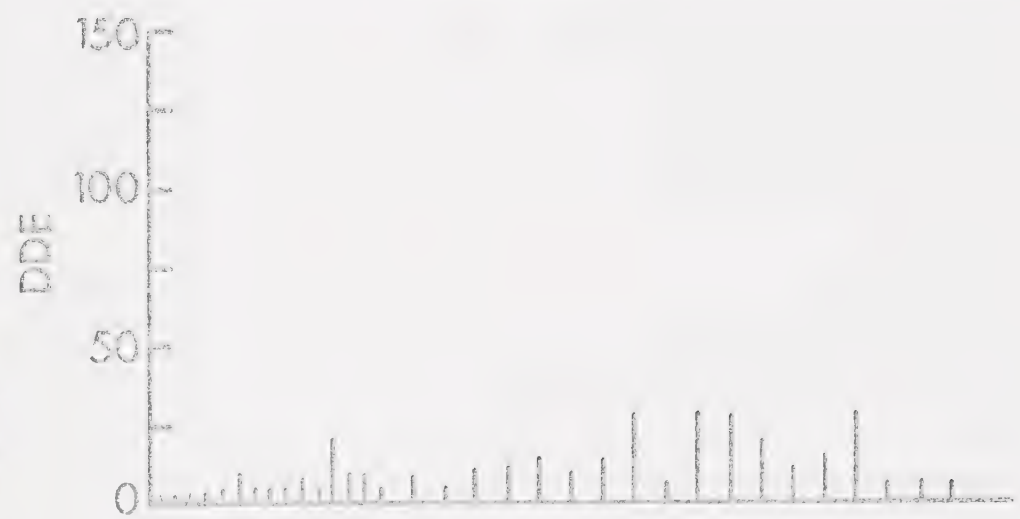
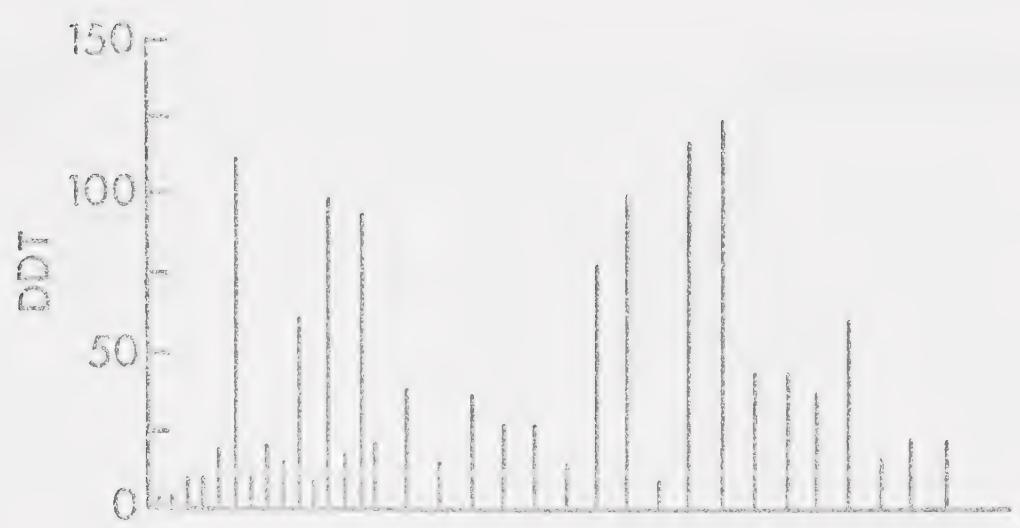


Figure 4. Residues of DDT, DDE and DDD in livers of DDT-treated birds sacrificed periodically during the study

RESIDUES IN LIVERS (ppm)



NUMBER OF DAYS AFTER STUDY INITIATION

available from more birds the trend in levels might have been similar to that seen for eggs (Figure 3). DDE was always present in less quantity than DDT and tended to parallel the level of DDT rather closely ($r = 0.767$). DDD levels correlated more strongly to levels of DDE ($r = 0.728$) than to DDT ($r = 0.608$) and although it always exceeded DDE in the liver DDD also in some birds exceeded DDT. The level of total residues in livers was significantly correlated to liver weights ($r = 0.57$), suggesting a maximum concentration per unit weight of liver tissue. The livers of the 10 control birds contained a low mean of 0.24 ppm of total residues with a standard deviation of 0.48 ppm. A typical gas chromatogram of liver residues is included in Figure 13.

DDT and Metabolite Residues in Fat

Figure 5 illustrates the residues found in the fat of the omentum of selected DDT-treated birds sacrificed during the study. As was the case for the residue levels presented in Figures 3, 4 and 6-11 some individual variation should be present. It is obvious that the residue levels of both DDT and DDE did not rise continually throughout the study and that a plateau level may have been established late in the study. DDT always exceeded DDE although the amount of DDE relative to DDT increased during the first 50 days of the study, but then remained relatively constant. Similar to the case of the egg extracts, DDD was present but was less than 1% of the DDT present and was not quantitated for the reason mentioned above. Total residues in the fat were not correlated to the weight of the omental fat ($r = -0.14$). The fat of a control bird sacrificed on day 6 contained only about 0.2 ppm of total residues. A typical gas chromatogram of the fat samples is presented in

Figure 5. Residues of DDT and DDE in omental fat of selected DDT-treated birds sacrificed during the study

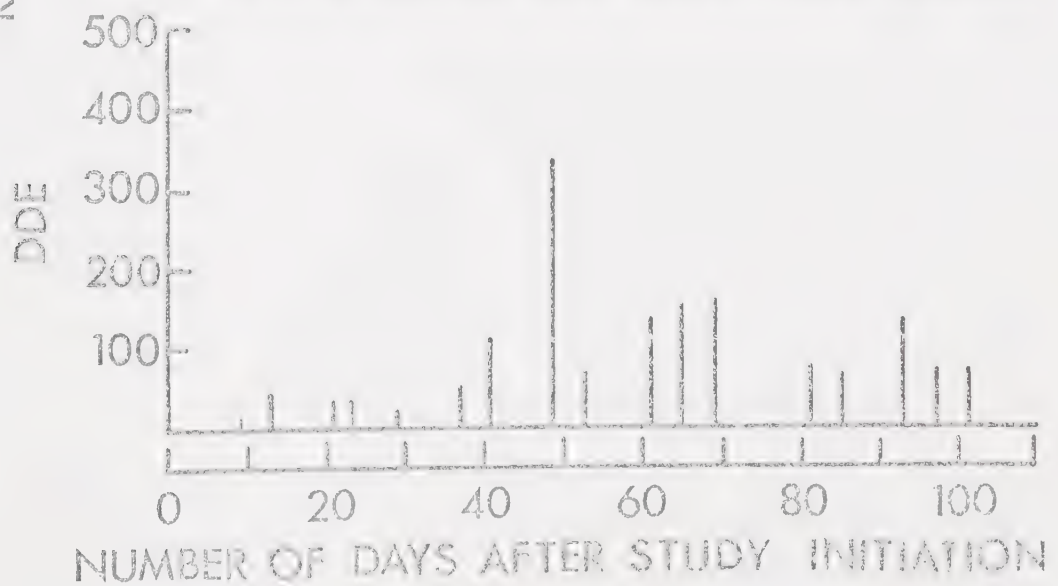
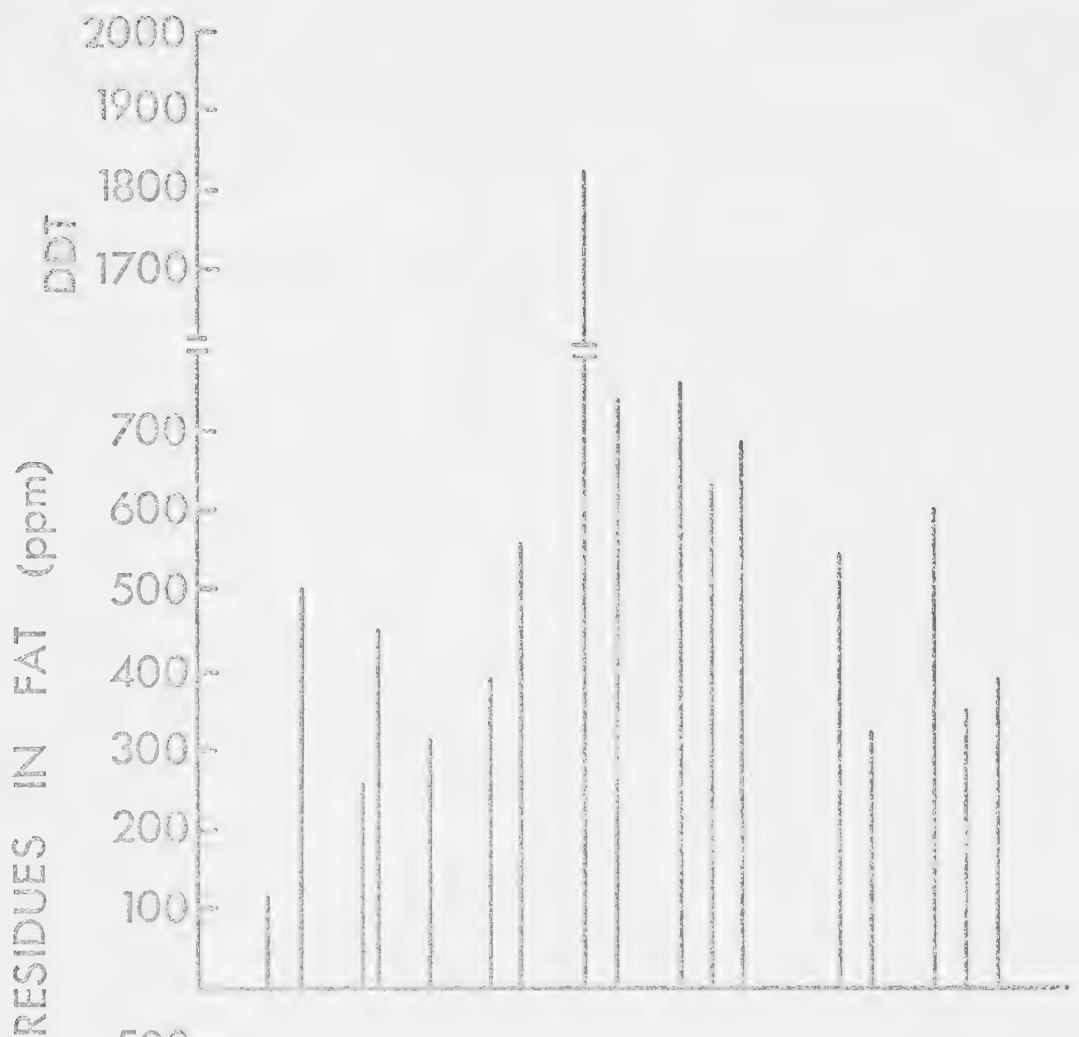


Figure 13. Correlations of total residue levels in the 14 fat samples to the total residues in livers from the same birds were not significant ($r = 0.34$).

DDT and Metabolite Residues in Uropygial Glands and Feces

Qualitative analyses of 6 uropygial glands indicated a predominance of DDE and DDT and only a relatively minute amount of DDD (Figure 13). These residues were also quantitated and will be presented below with individual bird parameters (Table 5). The feces residues were comprised of measurable quantities of DDD as well as DDE and DDT as can be seen in the chromatogram in Figure 13. Residues in samples of feces from 6 DDT-treated birds sacrificed late in the study were quantitated and these values were used in the calculations to be presented in Table 7.

Individual DDT-Treated Bird Parameters

Figures 6 to 11 and Table 5 display the results of investigations of several individual parameters of 6 DDT-treated birds sacrificed near the termination of the study. Graph (a) in Figures 6-11 shows the relationship of body weight and egg production to time. Although individual variations occur there are no cases of exceptional deviations from the group means of these parameters seen above. Similarly, the plots of DDT and DDE in individual eggs versus time (presented in part (b) of Figures 6-11) demonstrate the individual variations in levels of residues present, but when grouped with other egg residue data and presented as means (Figure 3) a general pattern can be discerned. Graph (c) of Figures 6-11 traces the trends of eggshell thickness versus time for individual birds' eggs. There is no obvious trend toward eggshell

Figure 6. Parameters of the DDT-treated bird of cage 37. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time.

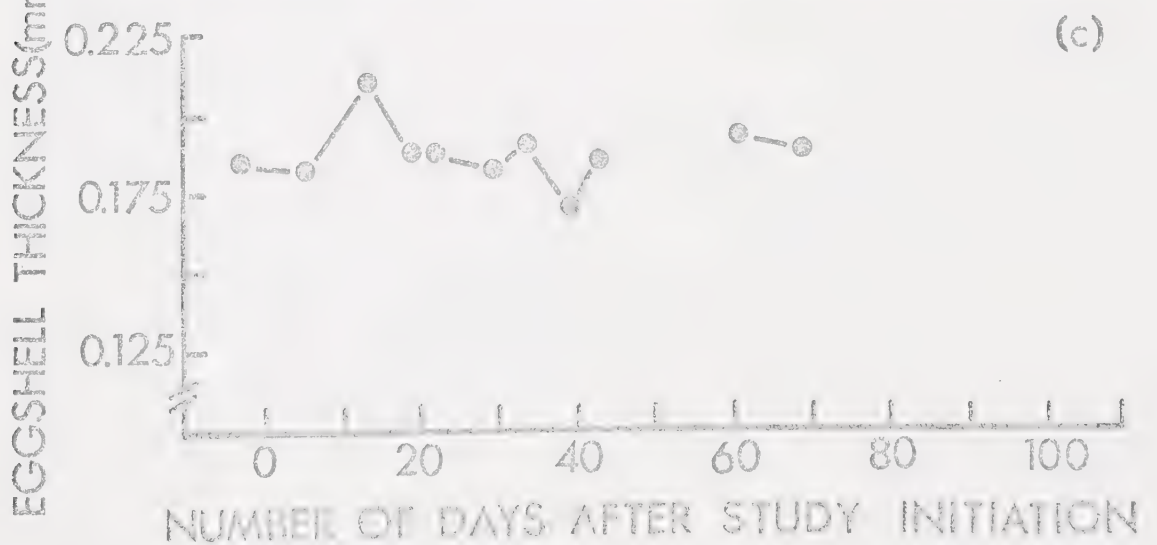
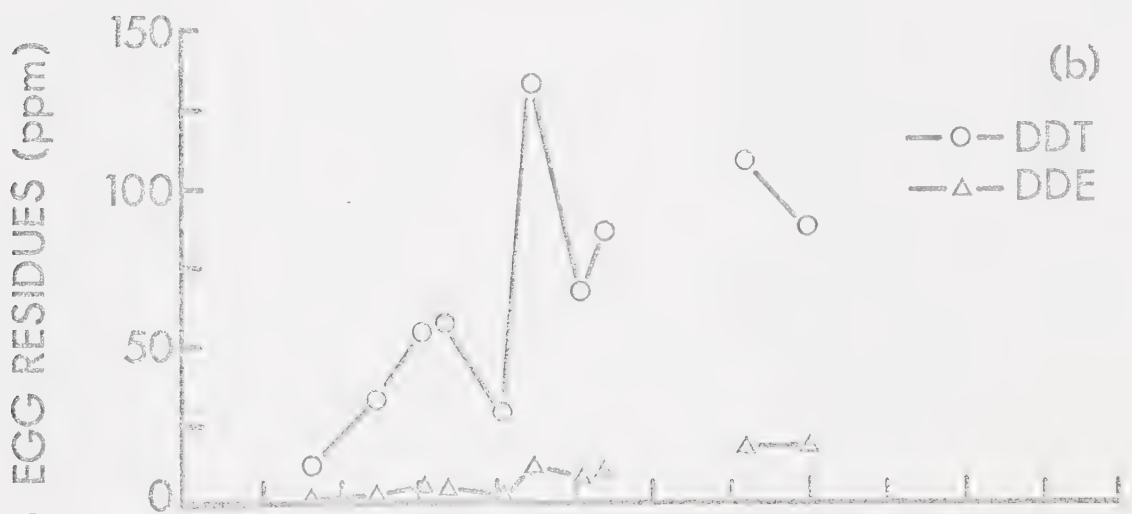
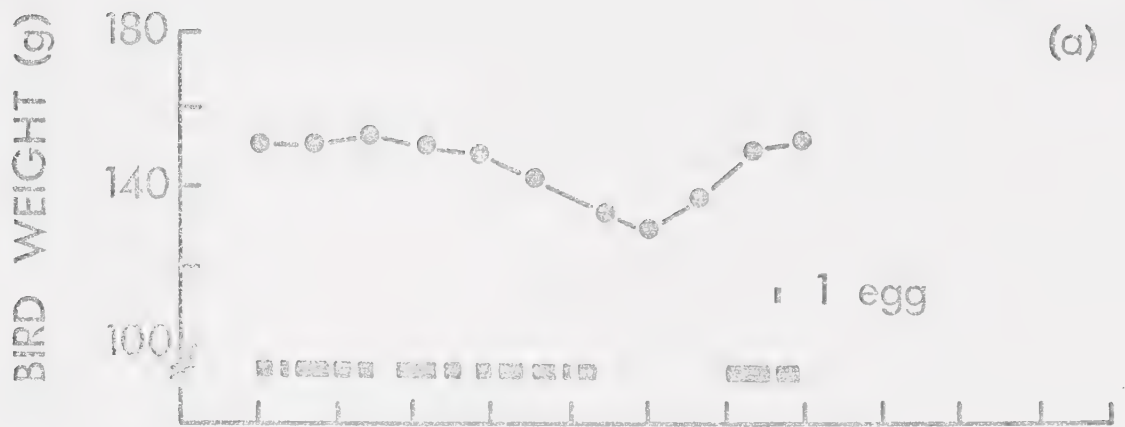


Figure 7. Parameters of the DDT-treated bird of cage 40. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time.

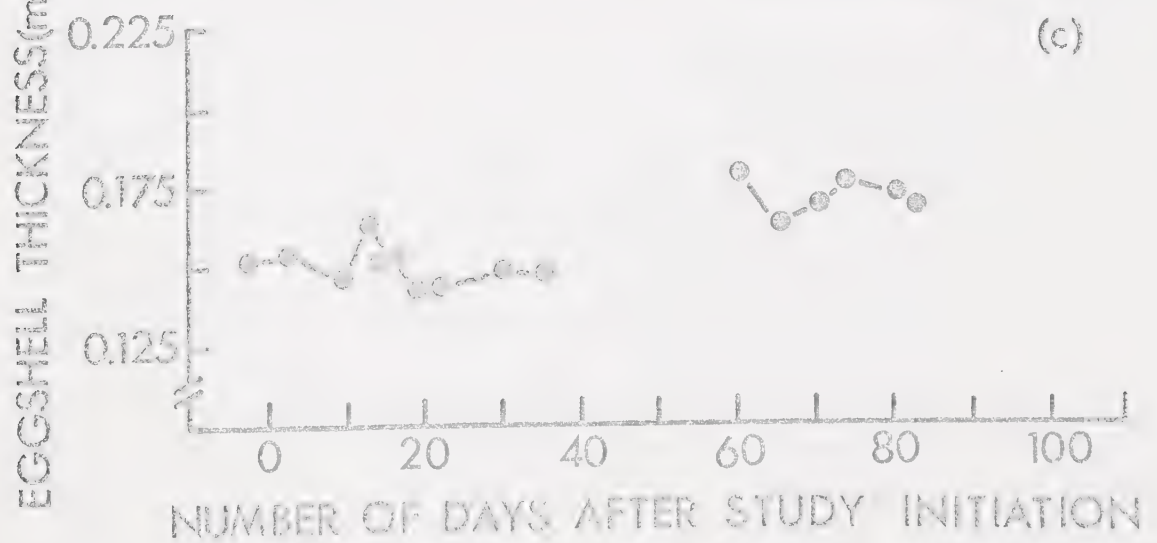
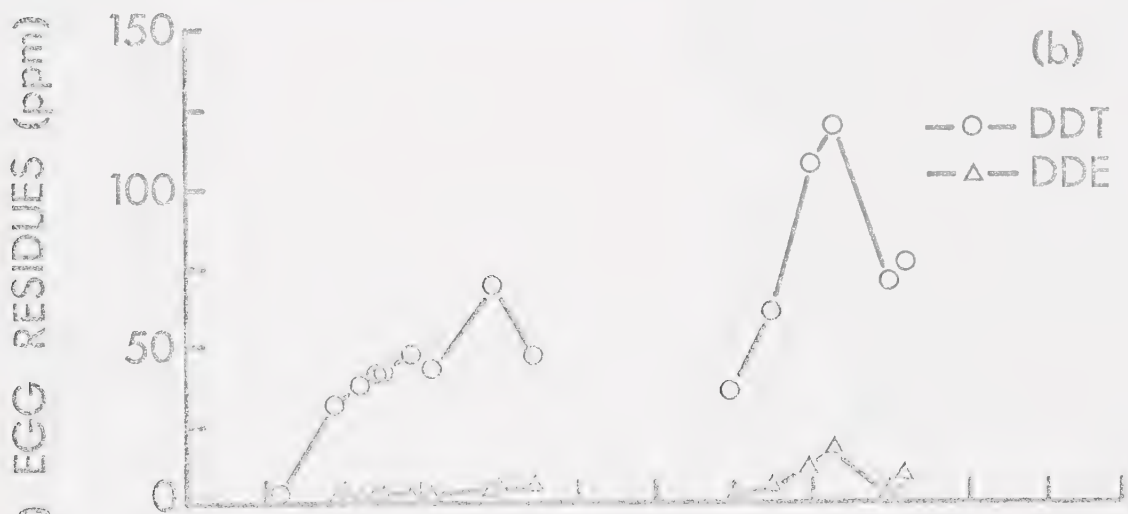
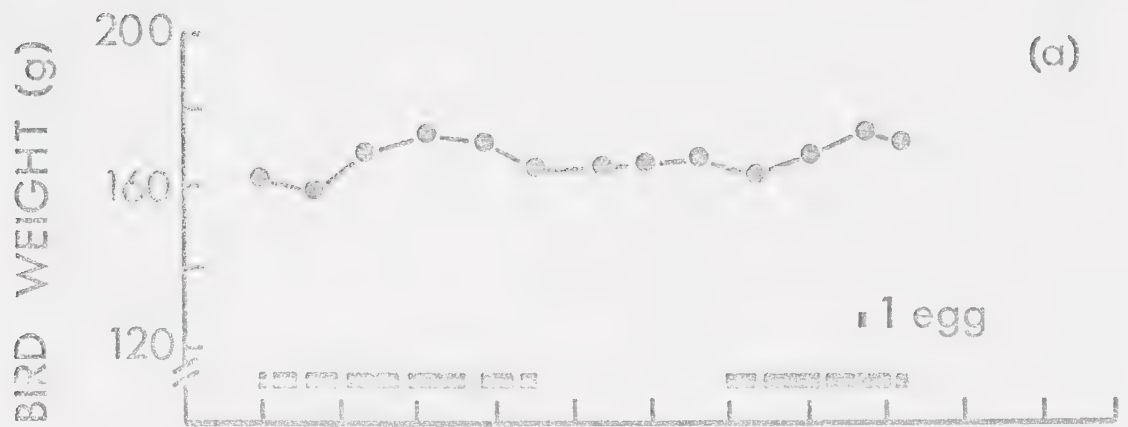


Figure 8. Parameters of the DDT-treated bird of cage 41. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time.

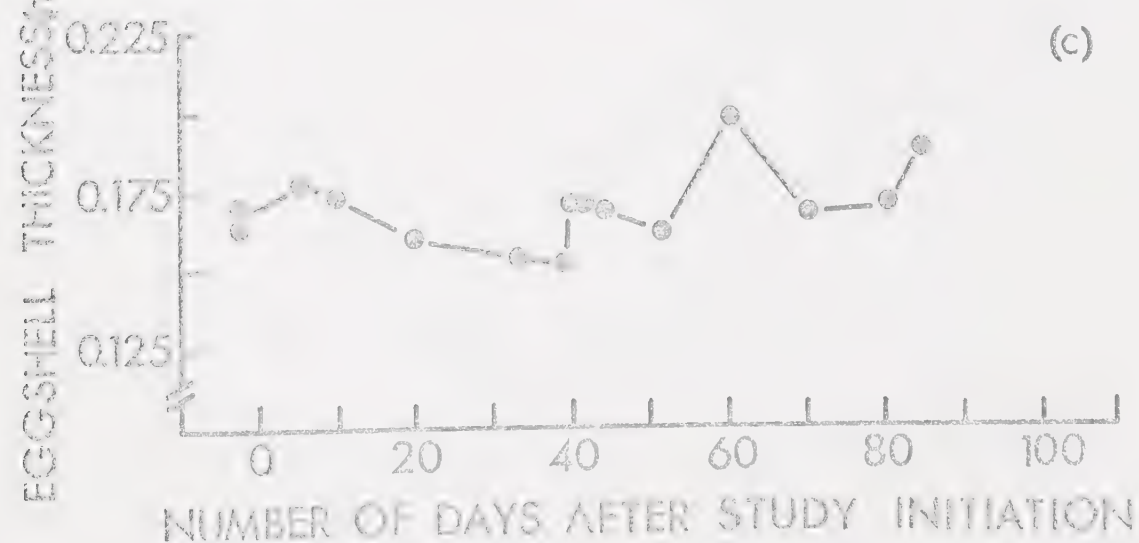
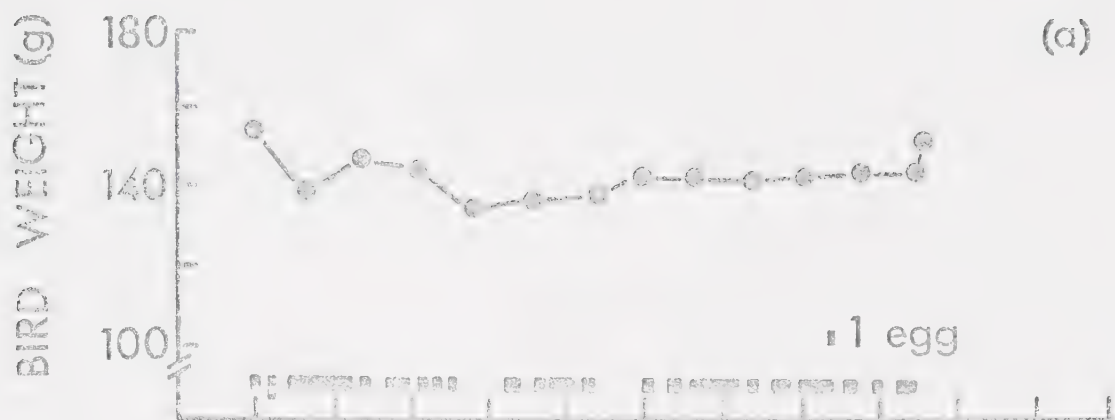


Figure 9. Parameters of the DDT-treated bird of cage 43. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time.

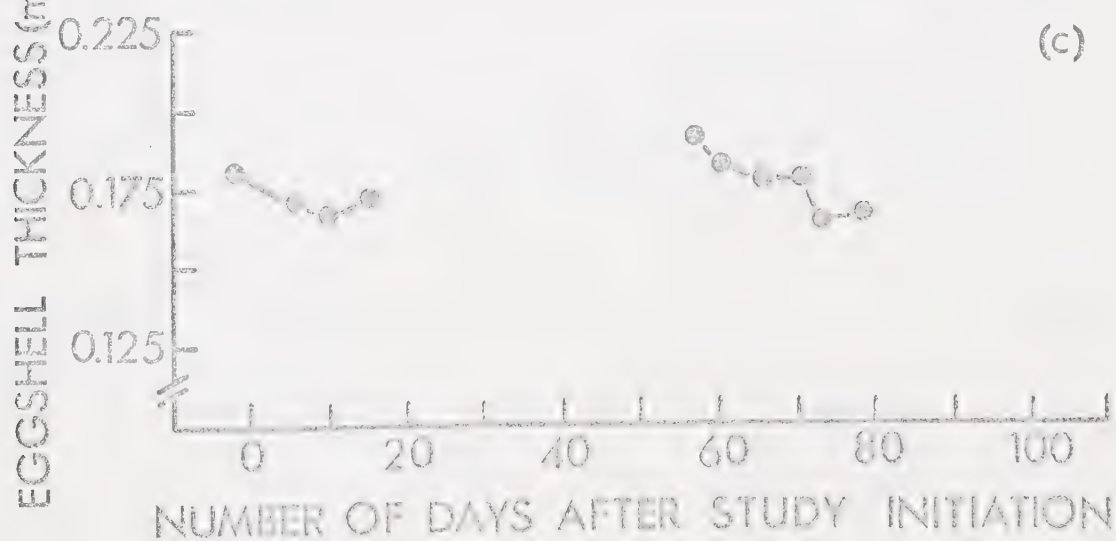
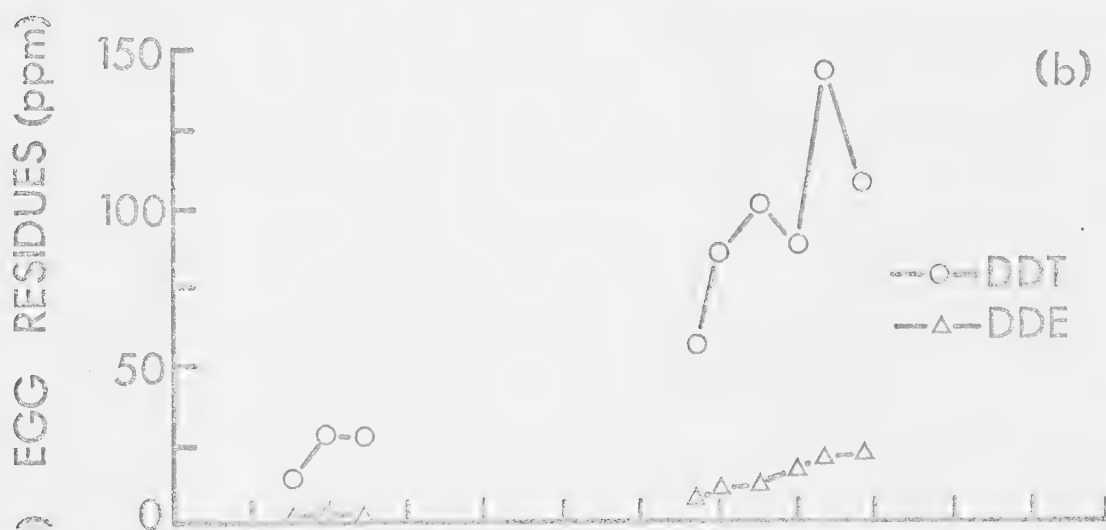
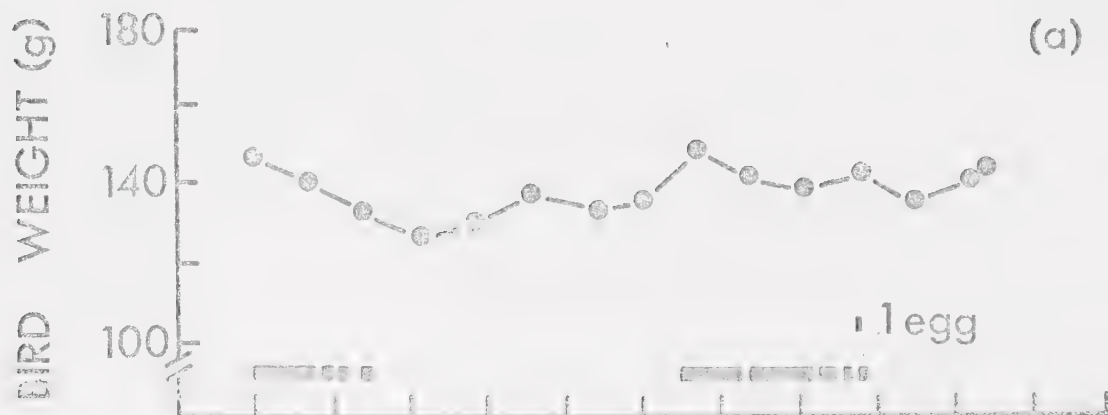


Figure 10. Parameters of the DDT-treated bird of cage 44. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time.

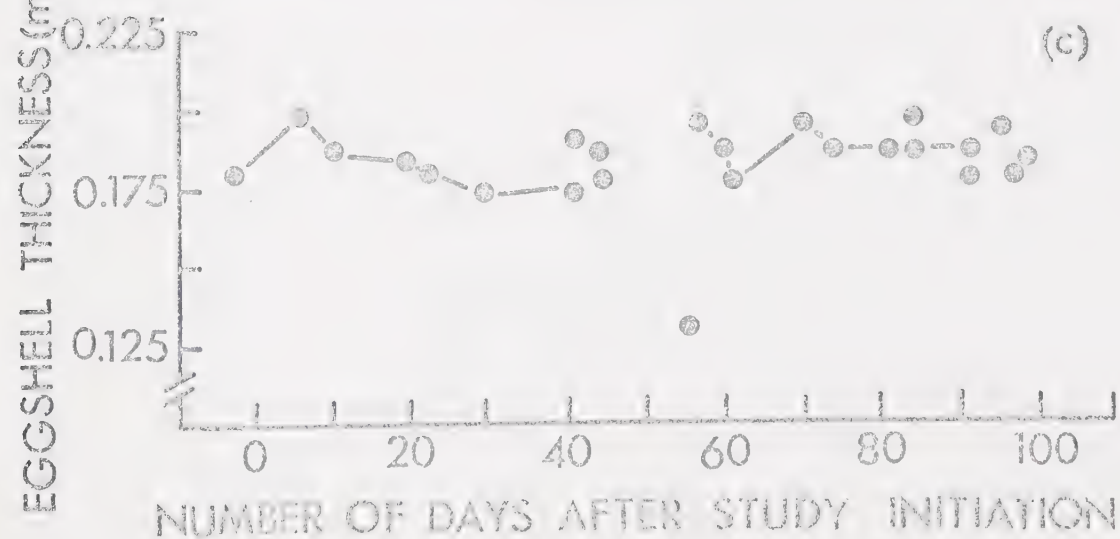
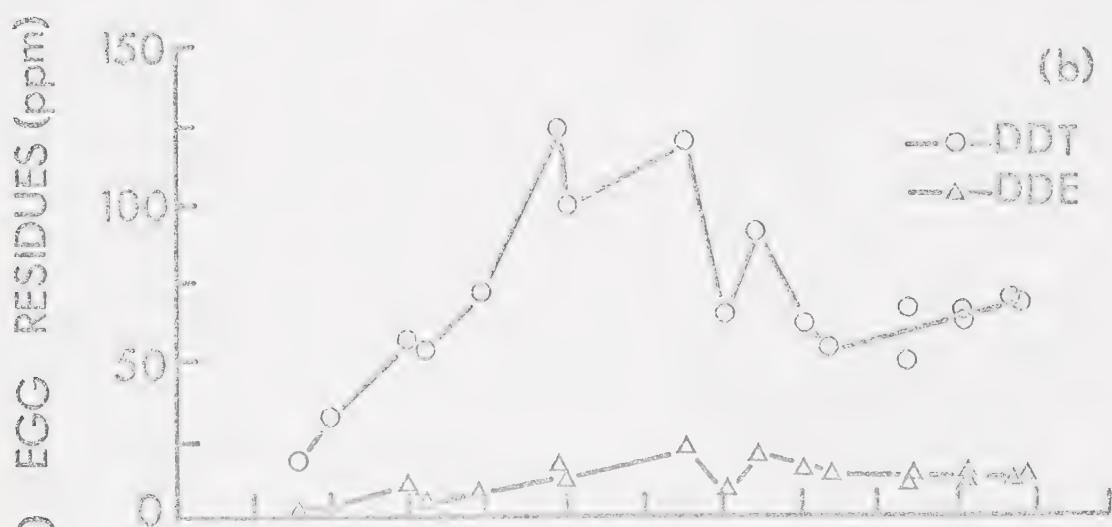


Figure 11. Parameters of the DDT-treated bird of cage 34. (a) Bird weight (upper plot) and egg production (lower plot in which each shaded half-square represents 1 egg) vs time. (b) DDT and DDE residues in eggs vs time. (c) Eggshell thickness vs time.

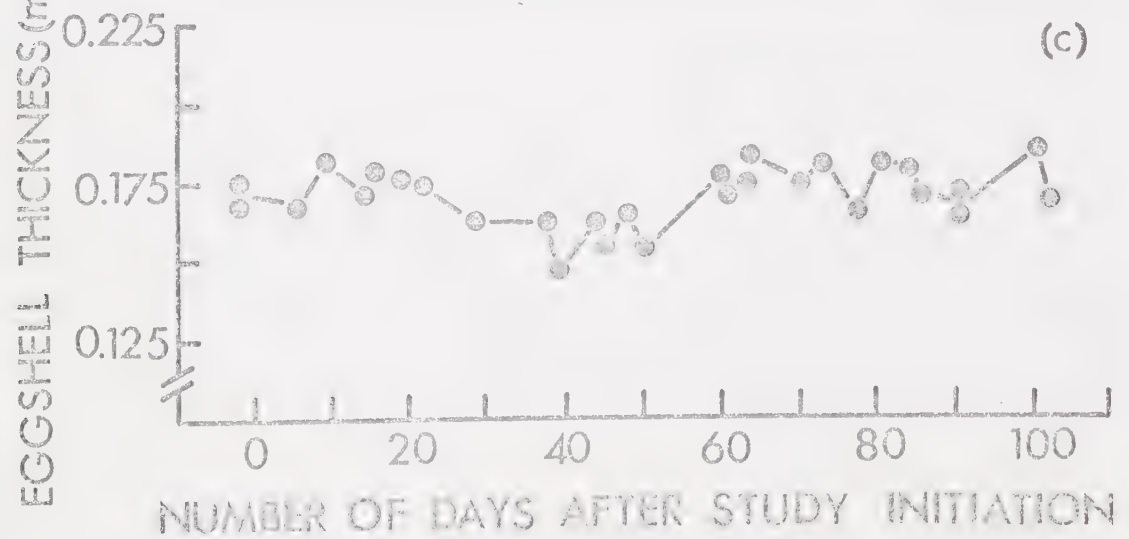
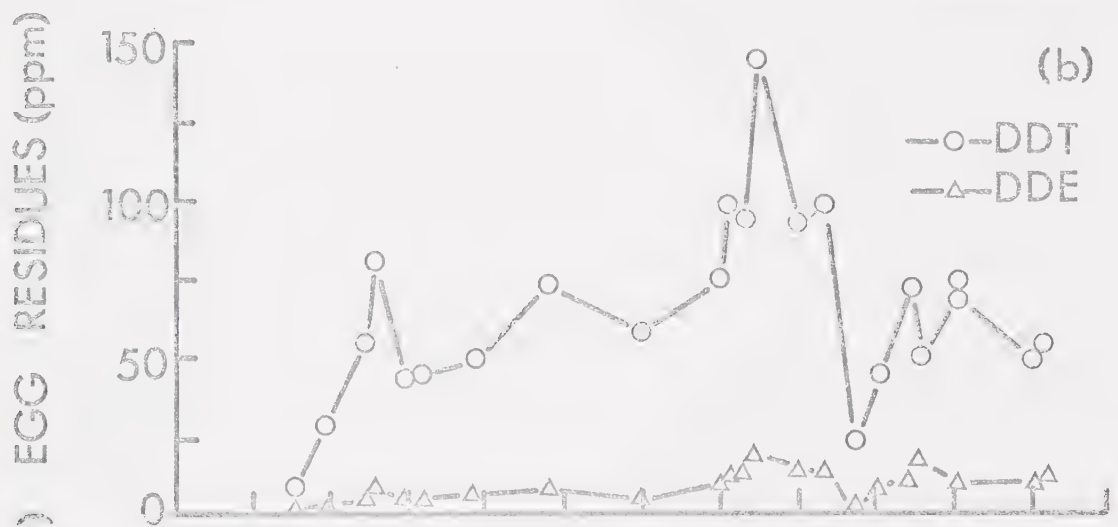
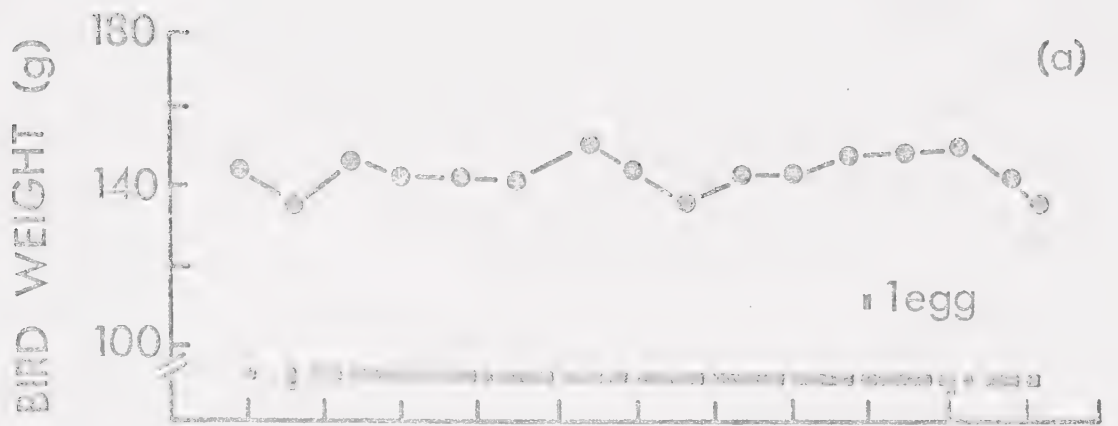


Figure 12. Eggshell thickness of the control bird of (a) cage 6 and (b) cage 10 plotted vs time.

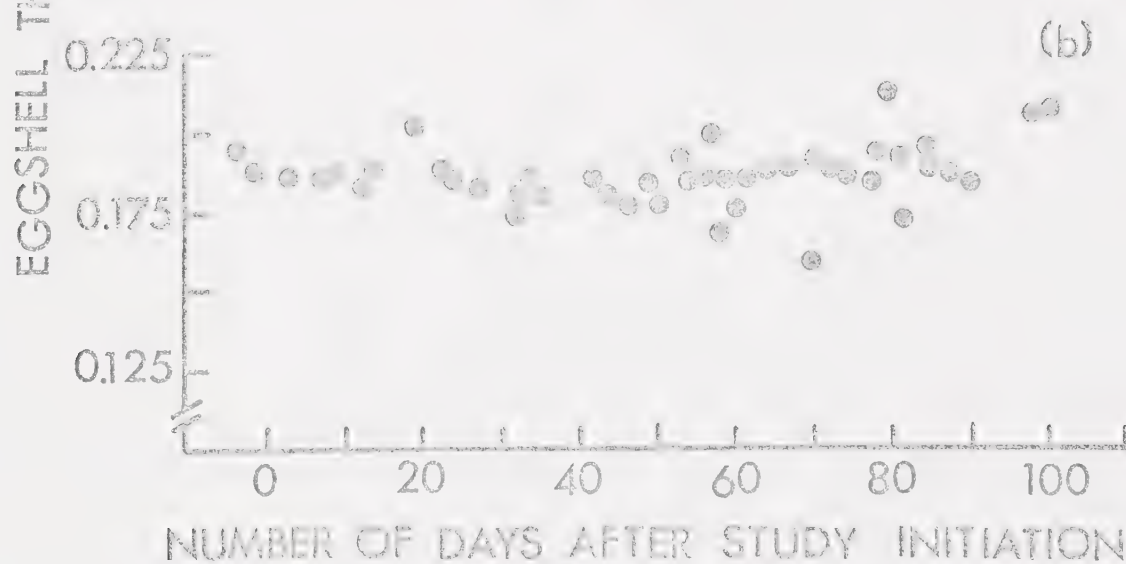
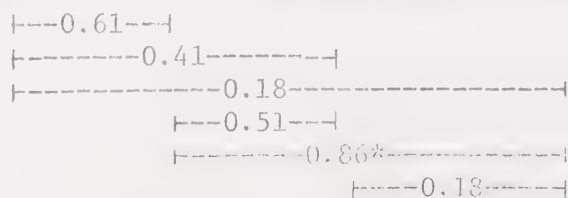


Table 5. DDD, DDE and DDD residue levels in livers, fat, uropygial glands and last eggs before sacrifice of DDT-treated birds of cages 37, 40, 41, 43, 44 and 34

Cage No.	Day of sacrifice	Chemical	Tissue residues (ppm)				Last egg before sacrifice
			Liver	Fat	Uropygial gland		
37	69	DDT	115	690	223		88
		DDE	28	159	60		18
		DDD	32		2		
		Total	175	849	285		106
40	81	DDT	42	549	212		78
		DDE	10	77	41		11
		DDD	16		5		
		Total	68	626	258		89
41	85	DDT	36	324	121		44
		DDE	13	63	27		9
		DDD	21		2		
		Total	70	387	150		53
43	93	DDT	15	606	154		107
		DDE	6	136	42		23
		DDD	12		3		
		Total	33	742	199		130
44	97	DDT	21	349	113		67
		DDE	6	66	27		11
		DDD	12		1		
		Total	39	415	141		78
34	101	DDT	20	391	246		54
		DDE	6	68	49		10
		DDD	13		3		
		Total	39	459	298		64

Correlation coefficients (r)
between tissues for
total residues



*Significant ($P < .05$).

thinning during the study and the plots are essentially the same as those for 2 control birds (Figure 12).

The main feature of the data presented in Table 5 was the variability of residue levels between the 6 birds. In all cases the total residues in fat exceeded those of uropygial glands and both exceeded those of the last egg before sacrifice (all within 2 days of sacrifice except that of cage 43 which was laid 15 days before sacrifice which may explain its high level of residues). The total level of residues in the liver never exceeded those of the fat or uropygial gland but exceeded that of the eggs in 2 of the 6 birds. There was only 1 correlation of total residues between the 4 sources which was significant, that of fat levels to egg levels ($r = 0.86$) though the small sample size probably adds bias to the data.

Fate of the DDT

In Table 6 the probable fate of DDT and its metabolites in 6 birds sacrificed near the end of the study is presented. The total amount of residues in mg was calculated for the livers and omental fat pad (referred to as fat in the table) on a whole weight basis. The total amount of residues excreted via the uropygial gland (U.G. in the table) was calculated on the basis that the gland could excrete a maximum of its own weight of oil per day. The total number of eggs and the residues in them (Figures 6-11) allowed simple calculation of the role of eggs in the excretion process. Subtracting the total of the known tissue residues plus the known excreted residues from the amount of DDT ingested gave the amount of DDT which must have been stored elsewhere in the body or excreted via the feces. Since other tissue residue concentrations

Table 6. Fate of total ingested p,p' -DDT in 6 DDT-treated birds sacrificed near the end of the study

Known residues of DDT and metabolites (mg)								
Cage No.	Day of sacrifice	In tissue		Excreted		Total	Total ingested (mg)	Stored or fecal DDT (mg)
		Liver	Fat	U.G.	Eggs			
37	69	1.2	0.6	3.9	26.4	32.1	92.5	60.4
40	81	0.5	2.0	6.7	33.1	42.3	119.1	76.8
41	85	0.4	1.3	3.2	17.2	22.1	113.9	91.8
43	93	0.1	2.4	3.1	28.3	33.9	124.6	90.7
44	97	0.3	0.3	3.0	50.1	53.7	117.4	63.7
34	101	0.1	1.5	7.1	51.2	59.9	135.3	75.4

were unlikely to have approached those of the omental fat it can be assumed that excretion of DDT and metabolites via the feces was high.

The balance between DDT intake and excretion for the 24-hr period prior to sacrifice of 6 DDT-treated birds late in the study is presented in Table 7. Again individual variation should be expected. For the bird of cage 37 the excreted residues exceeded the ingested DDT probably since this bird was sacrificed just as tissue residues were declining (Figures 3, 4, 5 and 6). For the other 5 birds the levels of detectable excreted residues were lower than the levels of DDT ingested. Even allowing for losses during cleanup and recovery the difference between ingested and excreted DDT and metabolites is marked.

Gas Chromatograms

Figure 13 simply illustrates typical examples of the chromatograms used for qualitative and/or quantitative analysis of the residues in the 5 substances listed. Retention times of the chemicals may vary somewhat between chromatograms due to slightly different operating conditions of the gas chromatograph. Measurement of peak heights to the nearest mm was only carried out if the heights were between 20 and 100 mm which was well within the 30% of the standing current where a linear detector response could be expected (Bonelli, 1966). Note again that while DDE and DDT are present in eggs, livers, fat, uropygial glands and feces, only the liver and feces contain appreciable levels of DDD.

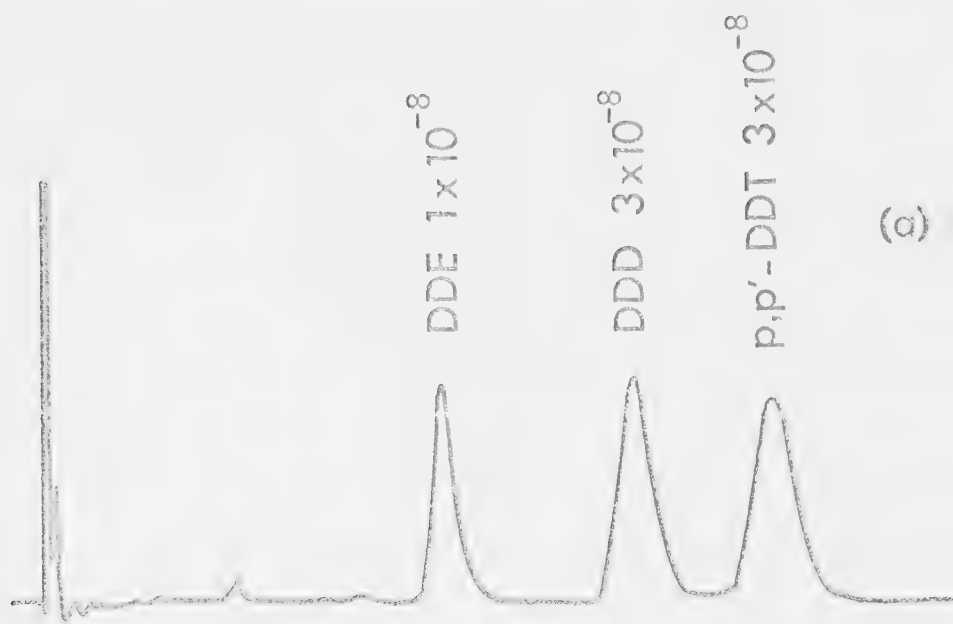
Bird Mortality

The only mortality during the study was that of a DDT-treated bird which died exhibiting the usual symptoms of DDT poisoning. The

Table 7. DDT intake and excretion for the 24 hr period prior to sacrifice of 6 DDT-treated birds near the end of the study

Cage No.	Day of sacrifice	DDT and metabolites excreted (mg)				DDT ingested (mg)
		U.G.	Egg	Feces	Total	
37	69	0.06	1.06	0.74	1.86	1.34
40	81	0.08	0.89	0.09	1.06	1.47
41	85	0.04	0.37	0.01	0.42	1.34
43	93	0.03	----	0.76	0.79	1.34
44	97	0.03	0.70	0.09	0.82	1.21
34	101	0.07	0.60	0.46	1.13	1.34

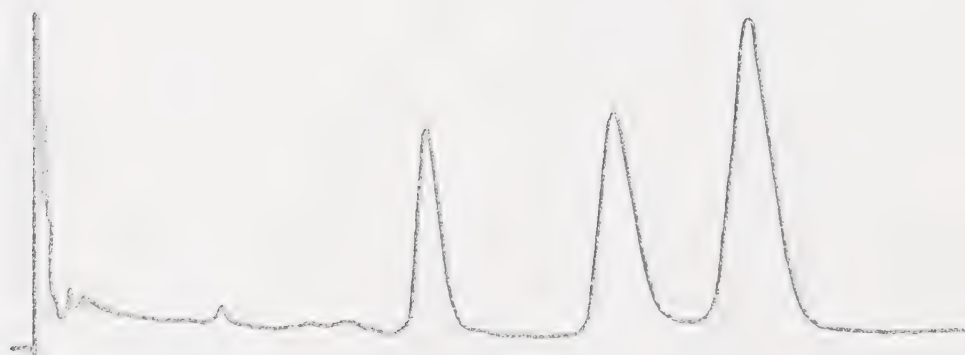
Figure 13. Typical gas chromatograms of (a) a 10^{-8} g/ml solution, (b) an egg extract, (c) a liver extract, (d) a fat extract, (e) a uropygial gland extract, and (f) a feces extract.



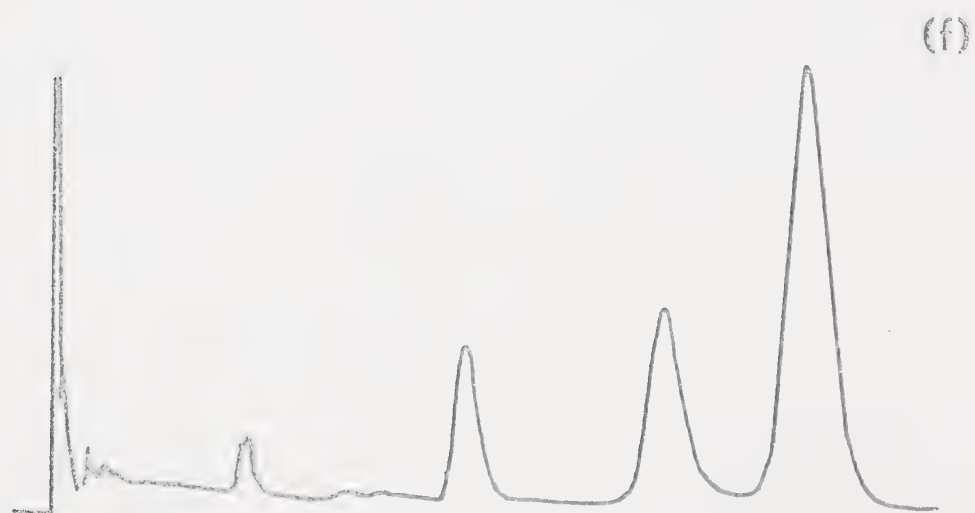
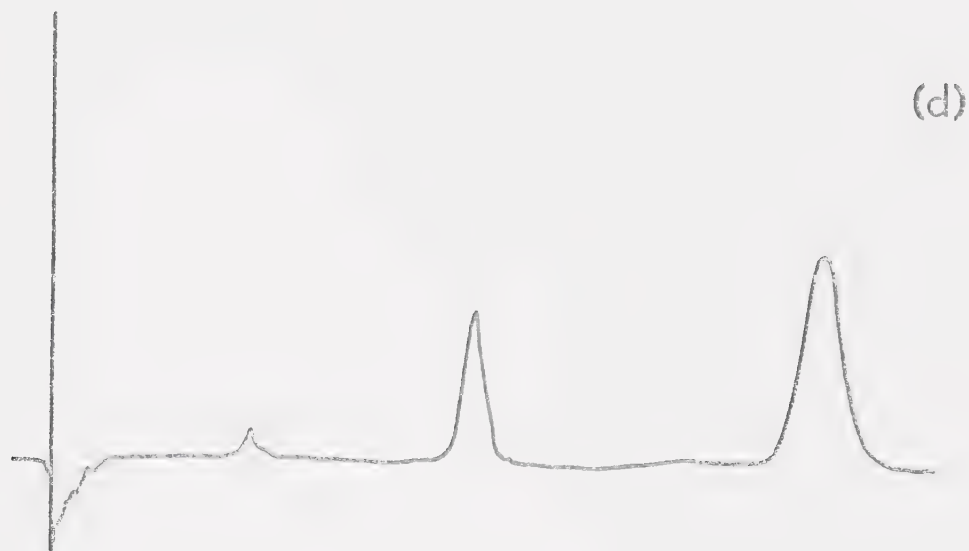
(a)



(b)



(c)



death occurred on day 48 during the period of apparent stress induced by the temperature and room change. The fact that this bird had not laid any eggs after the study initiation means that it could have accumulated higher insecticide residues than laying birds. General inactivity, a precipitous drop in body weight and an absence of body fat indicates that the bird had probably ceased eating and was metabolizing body fat stores for energy requirements. The liver contained less than 1 ppm of *p,p'*-DDT, 66.7 ppm DDE and 225.8 ppm DDD, a total liver pesticide load higher than that of any other bird examined. Although other factors could be involved this mortality indicates that this treatment of DDT is potentially lethal to non-laying birds under stress conditions. This bird may have died up to 24 hours before being dissected thus accounting for the preponderance of DDD in the liver since a postmortem conversion of *p,p'*-DDT to DDD in avian liver has been reported (Walker, 1969).

DISCUSSION

Live body weight could have acted as an index of the general health of the birds and was measured periodically for this reason. No differences arose between DDT-treated and control birds. Furthermore, the DDT-treated birds exhibited no drop in egg production and no increased incidence of moult when compared to control birds. Therefore, though DDT is a known toxicant it had no effect on these parameters indicating that any change in general health was at least not measurable using these simple indices.

The effect of the room change on the birds cannot be overlooked. Body weights and egg production dropped and an increased incidence of moulting occurred. Also, the only mortality of the study occurred during this period. These stress-induced changes, however, were parallel between control and DDT-treated groups with the exception of the mortality discussed above. Hence DDT during the period of stress had a potential lethal effect but apparently did not affect the birds otherwise.

As expected the p,p' -DDT was at least metabolized to DDD and DDE with the liver acting as the probable site of the transformations. It was thought that the livers of DDT-treated birds might increase in size and weight to facilitate these functions under the influence of a chronic dose of DDT. The weight change did not occur.

Use of such indicators as body and organ weights could yield evidence of gross abnormalities caused by an agent such as DDT but negative results do not necessarily imply that there was no effect.

Subtle changes or changes in other organs and physiological functions not examined would obviously not be discovered. Therefore, although the gross parameters measured in this study are of value their meaning is limited.

The fact that DDT did not affect egg production during this study was not surprising since it had previously been reported by Cross *et al.* (1962), Ernst (1966), Smith *et al.* (1969) and Cecil *et al.* (1971). Also in support of the findings of Cross *et al.* (1962), Chang (1970) and Cecil *et al.* (1971) DDT in the present investigations caused no change in egg weights. And although the lack of eggshell thinning in DDT-treated birds in this study disagrees with the earlier findings of Bitman *et al.* (1969) it is in agreement with their recent findings (Cecil *et al.*, 1971).

Thus, the relatively high dose of *p,p'*-DDT and resultant high levels of DDE in birds of this study caused no changes in the above-mentioned reproductive parameters. Yet DDT and/or DDE have been implicated as the cause of reproductive failures in several wild avian species. This discrepancy could be explained simply as a variable response to the insecticide by different species. It is also important to consider that wildlife studies have never 'proven' a cause and effect relationship between DDE and reproductive failure. The implications have been correlative and as such are based on logic. That is, it is not impossible that although DDE is present some other factor(s) is (are) causing the reported effects. The present study reports only that DDT and DDE did not affect three reproductive parameters of Japanese quail and does little to clarify the meaning of reports from similar environmental investigations.

Total DDT and metabolite residues in eggs, liver and fat exhibited a rise early in the study, a mid-study approximation of a maximum level and a late-study indication of a reduced level. From this it is clear that DDT at a given fixed intake did not indefinitely magnify in the tissues of these birds. It would appear in fact that the birds became adapted to the intake and by the time some maximum tissue concentration had been reached certain metabolic processes had been induced to reduce the tissue concentration of residues and possibly stabilize them at a level proportional to the intake.

This finding supports the idea of a plateau level of residues for a given DDT intake discussed earlier and is in agreement with the report of Wright *et al.* (1972). That is, from this study it appears that biological magnification of DDT from a given chronic source occurs only to a point beyond which a plateau of tissue levels is observed. This, however, may not necessarily be true for all species and all diet contamination levels so broad generalizations are best to be avoided. For instance, if one assumes accurate quantitation, the high levels reported in some wildlife studies (where the DDT input is generally low) may be the result of no plateau level forming in some species, or a relatively high plateau level being accumulated. Furthermore, the number of samples examined is important since individual variations should be expected.

The presence of DDD and DDE as well as p,p' -DDT in the livers suggests that the conversions of p,p' -DDT to DDE and DDD occur there or in the gut prior to absorption. The liver, supplied by the hepatic portal system, is the probable site of metabolism since it is known to carry out many similar and varied metabolic functions. Whether some microbial

conversions of DDT to metabolites occurs in the gut is speculative. The presence of DDD only in livers and feces in appreciable quantities suggests that it is entirely formed in the gut or liver but is limited to these sites or if generally present and transported in blood it is not concentrated by eggs, fat or uropygial glands as are DDT and DDE. DDE is present in all substances examined supporting the idea that it is the most commonly detected avian metabolite of DDT.

Correlations of DDT and residue levels between different tissues in the same bird show a general pattern but the relationships are not rigid. Fat concentrates more DDT and DDE than does the uropygial gland and levels in both exceed those of both the livers and eggs. Such correlations between tissues should be strongest during the portion of the study when the plateau level has been reached and for this study these investigations were carried out on 6 birds which were sacrificed near the termination of the experiment. Still only correlations of fat levels to egg levels were highly significant. Predictions of other tissue levels on the basis of egg residue levels can obviously be made only with some degree of error due to the amount of individual variation apparent within this species.

One other factor could affect tissue residue level correlations. It is possible that administering the total dose of DDT at one time during the day is not an ideal technique. It does not parallel an environmental situation and the amount absorbed and/or excreted may vary somewhat with the time of day. The method of DDT administration by capsule does avoid the problem of differential food ingestion inherent in feeding studies where DDT is added to the feed. However, even for the capsule method

reported here it cannot be assumed that the dose is strictly identical from bird to bird due to the possibility of a variability in the rate of capsule digestion and DDT absorption. Differences might also be expected in the liver and enterohepatic pathway activities and would be reflected in tissue residue levels. Although these are unavoidable and uncontrollable variables they may help explain some of the results presented above.

For instance, on the basis of this discussion residue levels in livers would be expected to vary more from bird to bird because: the livers are near the gut and receive the insecticide more or less directly; the liver is the probable metabolic centre for DDT and levels present there will reflect variations in liver activity between individual birds. Eggs and tissues such as fat and uropygial glands which acquire their residue levels more indirectly and over a longer time period should give a more uniform pattern of residues even allowing for individual variability of metabolism. Data from this study with regard to residue levels in livers, eggs and fat lend support to these theories.

The data concerned with the fate of DDT ingested during the study revealed that the majority of this DDT was excreted either directly in the feces or indirectly in the feces and eggs.

A declining or stationary level of both DDE and DDT in eggs and fat and of DDE, DDD and DDT in liver late in the study simply means that an amount of DDT and metabolites equal to the DDT ingested is being excreted or metabolized to undetected compounds. The relatively low levels of DDT, DDD and DDE found in the feces of the last 5 DDT-treated birds sacrificed suggest excretion of some of the *p,p'*-DDT as undetected metabolites.

SUMMARY

A capsulated dosage of 9 mg/kg (based on body weight) of *p,p'*-DDT was administered daily to 34 Japanese quail over a period of 101 days. The following results were observed:

1. The DDT had no apparent effect on the general health of the birds as indicated by body weight, egg production, incidence of moult and liver weights.

2. The reproductive parameters of egg production, egg weight and eggshell thickness were not altered by the treatment of DDT or the resultant DDT and metabolite residue buildup in tissues and eggs.

3. The *p,p'*-DDT was converted to DDD and DDE probably within the livers of the birds. DDT and DDE were present in eggs, livers, fat, uropygial glands and feces. DDD was also present in appreciable quantities in livers and feces.

4. DDT and metabolite residue levels in eggs, livers and fat exhibited a rise early in the study, a mid-study approximation of a maximum level and a late-study indication of a reduced level. That is, DDT residues were not continuously magnified in the tissues examined.

5. Residue levels in fat tissues exceeded those of uropygial glands and levels in both of these tissues were greater than those in eggs and livers. Liver residue levels varied considerably from bird to bird and showed no obvious relationship to egg levels.

6. Correlations between tissue and egg residue levels were tested for 6 birds sacrificed late in the study. Only the correlation of fat residues to egg residues was significant.

7. Declining or stationary levels of residues in eggs, livers and fat late in the study indicated that metabolism and/or excretion of the DDT and metabolites was equal or greater than the ingestion of DDT. Relatively low levels of DDT, DDD and DDE were present in the eggs, uropygial glands and feces of the last 5 birds sacrificed in the study and the total of these levels was less than that of the DDT being ingested daily. This suggested some excretion via undetected metabolites.

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APPENDIX 1

List of companies supplying chemicals, equipment
and materials for this study

Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, U.S.A.
Bioscience Animal Services, University of Alberta, Edmonton, Alberta.
Canadian Liquid Air Ltd., Edmonton, Alberta.
Chesebrough-Ponds (Canada) Ltd., Markham, Ontario.
Chromatographic Specialties Ltd., Brockville, Ontario.
Eli Lilly and Company, Indianapolis, U.S.A.
Federated Co-operatives Ltd., Edmonton, Alberta.
Fisher Scientific Company Ltd., Edmonton, Alberta.
Hamilton Company Inc., Whittier, California, U.S.A.
Ivan Sorvall Inc., Newtown, Connecticut, U.S.A.
Mallinckrodt Chemical Works Ltd., Toronto, Ontario.
Manostat Corporation, New York, New York, U.S.A.
Polyscience Corporation, Evanston, Illinois, U.S.A.
Purex Corporation Ltd., Weston, Ontario.
Rinco Instrument Company Ltd., Greenville, Illinois, U.S.A.
Valley Granite Products, Chilliwack, British Columbia.
Varian Aerograph, Walnut Creek, California, U.S.A.

APPENDIX 2

Formula used to calculate tissue residues in parts per million (ppm) or milligrams per kilogram (mg/kg):

$$\text{ppm} = \frac{\frac{\text{unknown peak height (mm)}}{\text{standard peak height (mm)}} \times \frac{\text{standard injection volume (ml)}}{\text{standard concentration (g/ml)}} \times \text{multiple of } 10^{-6} \text{ correction factor}}{\frac{\text{tissue weight (g)}}{\text{extract dilution (ml)}} \times \text{unknown injection volume (ml)}}$$

Formula derivation:

1. A linear relationship exists between peak height on the chromatogram and the amount of chemical injected.

2. Therefore, a ratio of the peak height of the unknown to the peak height of the standard will indicate how much unknown was present in the volume injected. Correction for the extract dilution and tissue weight will give the weight of chemical present in the tissue on a unit weight basis (ppm or mg/kg).

3. Let h = unknown peak height (mm)
 y = standard peak height (mm)
 z = extract dilution
 w = tissue weight (g)
 s = standard injection volume (ml)
 u = unknown injection volume (ml)
 k = concentration of chemical in unknown injection (g/ml)
 $c \times 10^{-8}$ = standard dilution (g/ml)

4. Amount of standard chemical injected =

$$s(\text{ml}) \times c \times 10^{-8} \text{ (g/ml)} = s \times c \times 10^{-8} \text{ (g)}$$

Amount of unknown chemical injected =

$$u(\text{ml}) \times k(\text{g/ml}) = u \times k(\text{g})$$

From above (2) we know:

$$\frac{h(\text{mm})}{y(\text{mm})} = \frac{u \times k(\text{g})}{s \times c \times 10^{-6}(\text{g})}$$

$$\text{i.e., } k = \frac{h}{y} \times \frac{s}{u} \times c \times 10^{-8}$$

Also from above (2) we know:

$$\text{ppm or mg/kg} = \frac{k(\text{g/ml}) \times 10^Z(\text{ml})}{w(\text{g})} \times 100 = \frac{k \times 10^Z}{w} \times 100$$

(A factor of 100 is necessary to correct for the 10^{-8} dilution of the standard thus changing the final answer to a multiple of 10^{-6} which of course is ppm.)

Substituting for k we get:

$$\text{ppm} = \frac{\frac{h}{y} \times \frac{s}{u} \times c \times 10^{-8} \times 10^Z}{w} \times 100$$

Rearranging we get:

$$\text{ppm} = \frac{\frac{h}{y} \times s \times c \times 10^{-8} \times 100}{\frac{w}{10^Z} \times u}$$

Substituting for the symbols according to 3 we get the desired formula.

APPENDIX 3

Analysis of quail feed

The feed used throughout this study was "26% Turkey Starter (Med. Polystat)" for which its producer, Federated Co-operatives Ltd., Edmonton, Alberta, gives the following analysis:

Crude protein	min. 26	%
Crude fat	min. 3.0	%
Crude fibre	max. 6.0	%
Salt	act. .35	%
Calcium	act. 1.3	%
Phosphorus	act. 1.0	%
Added Zinc	act. .0055	%
Synthetic Vitamin A	min. 4500	I.U./lb.
Synthetic Vitamin D ₃	min. 1200	I.U./lb.

"Medicated with acetyl(*p*-nitrophenyl)sulfanilamide - 0.03%, dibutylin dilaurate - 0.02%, dinitrodiphenylsulfonylethylene-diamine - 0.02%, 3-nitro-4 hydroxyphenylarsonic - 0.0075% (from Polystat) as an aid in: the prevention of coccidiosis, large roundworms and tapeworms in turkeys and in preventing hexamitiasis and non-specific enteritis (Bluecomb) in turkeys."

APPENDIX 4

The modified cleanup method of Langlois-Stemp-Liska (Bonelli, 1966)

1. 60/100 mesh Florisil (Fisher Scientific Co.) previously activated at 1200° C was reactivated at 150° C or 300° C for a minimum of 12 hours in a loosely capped quart sealer.

2. The Florisil was partially deactivated with 3% redistilled water by weight and kept in an airtight rubber-sealed quart sealer until used.

3. 50 g of Florisil was placed in the column and prewashed with 100 ml of a 50:50 methylene chloride:redistilled petroleum ether (B.P. 38.0° C-49.6° C) (Fisher Scientific Co.) solution.

4. 5 g of the sample was added to an additional 50 g of Florisil and ground in a mortar until a free-flowing powder resulted. This was then added to the top of the column above the previous 50 g of Florisil.

5. Two consecutive 100-ml portions of a 1:4 mixture of methylene chloride:petroleum ether were used to rinse the glassware and utensils and along with a further 400 ml of this mixture were added to the reservoir and allowed to elute from the column.

6. The eluate was flash evaporated to dryness using a Rinco rotary evaporator and a water bath maintained at 35-45° C.

7. 10 ml of spectroanalyzed n-hexane (Fisher Scientific Co.) was added to the flask and the resulting solution placed in a labelled screw-cap test tube.

8. The test tubes were stored in a freezer at -15° C until gas chromatographic analyses were carried out.

APPENDIX 5

On-column extraction-cleanup method for animal fat (Cahill *et al.*, 1970)

1. Up to 0.25 g of frozen fat was shaved onto aluminum foil using a scalpel and weighed to the nearest mg.

2. The fat was transferred to a 4" activated Florisil column which had been capped with 0.5" of sodium sulfate and prewetted with 50 ml of hexane. The foil was then reweighed to determine the actual weight of fat used.

3. The column walls were rinsed with 25 ml of hexane and the fat sample allowed to dissolve in this rinse for 5 min. The 25 ml was then eluted at 1 drop/3 sec.

4. When the previous rinse had entered the column bed the column walls were rinsed 3 times with 5 ml volumes of hexane.

5. The column was then eluted with 200 ml of 5% ethyl ether in hexane at 1 drop/sec.

6. Steps 6-8 of Appendix 4 were then carried out.

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